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=> d stat que
L1 3 SEA FILE=REGISTRY (BUSULFAN/CN OR "BUSULFAN MANNITOL"/CN OR "BUSULFAN-METHOTREXATE MIXT."/CN)
L2 1 SEA FILE=REGISTRY "CTLA4 (ANTIGEN) (SWINE GENE CTLA4 PRECURSOR) "/CN
L3 1139 SEA FILE=HCAPLUS L1 OR BISULFAN?
L4 298 SEA FILE=HCAPLUS L2 OR CTLA4IG OR CTLA41G
L6 22 SEA FILE=HCAPLUS (L3 OR L4)(L)(PANCREA? OR ISLET?)

=> d ibib abs hitrn l6 1-22

L6 ANSWER 1 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:580613 HCAPLUS
DOCUMENT NUMBER: 137:134555
TITLE: Pancreatic toxicity after liposomal amphotericin B
AUTHOR(S): Stuecklin-Utsch, A.; Hasan, C.; Bode, U.; Fleischhack, G.
CORPORATE SOURCE: Department of Pediatric Haematology-Oncology, University of Bonn, Germany
SOURCE: Mycoses (2002), 45(5-6), 170-173
CODEN: MYCSEU; ISSN: 0933-7407
PUBLISHER: Blackwell Verlag GmbH
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Though liposomal amphotericin B has been available in Germany since 1992, efficacy and safety of this formulation of amphotericin B are still not well-documented in children. As far as gastrointestinal side-effects are concerned, an elevated alk. phosphatase and elevated transaminases have been reported. In our department, liposomal amphotericin B had been used since 1994 to treat patients with proven or suspected fungal infections in

a daily dose of 1-3 mg kg-1. Addnl., patients with high-dose chemotherapy and autologous stem cell support received liposomal amphotericin B prophylactically in a dose of 1 mg kg-1 three times per wk. We performed a retrospective anal. of all 31 patients who had received liposomal amphotericin B by 1999. In five patients, an isolated transient elevation of the serum lipase level during, or shortly after, the therapy with liposomal amphotericin B was detected. Three of these patients showed clin. signs of pancreatitis, with one patient displaying slightly elevated transaminases. So far, elevated levels of serum lipase have not been described as a possible side-effect of a liposomal amphotericin B therapy. The pathogenesis of this elevation is unclear. As possible reasons, an enzyme induction due to fat overload or a toxic damage of the pancreatic tissue by the liposomes or amphotericin B itself are discussed.

IT **55-98-1**, Busulfan
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(**pancreatic** toxicity after liposomal amphotericin B)

REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 2 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:884097 HCAPLUS
DOCUMENT NUMBER: 136:165911
TITLE: Without CD4 help, CD8 rejection of pig xenografts requires CD28 costimulation but not perforin killing
AUTHOR(S): Zhan, Yifan; Brady, Jamie L.; Sutherland, Robyn M.; Lew, Andrew M.
CORPORATE SOURCE: Walter and Eliza Hall Institute of Medical Research, Parkville, 3050, Australia
SOURCE: Journal of Immunology (2001), 167(11), 6279-6285
CODEN: JOIMA3; ISSN: 0022-1767
PUBLISHER: American Association of Immunologists
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Although CD4 cells are major mediators in cellular rejection of fetal pig **pancreas** (FPP) in the mouse, rejection still occurs in the absence of CD4 cells, albeit with delayed kinetics. CD4 cell-independent mechanisms of cellular rejection are poorly understood. To investigate the involvement of CD8 T cells in FPP rejection and their activation requirements, we used mice transgenic for anti-CD4 Ab; this is the most complete model of CD4 cell deficiency. We showed that in such mice FPP was infiltrated with CD8 cells starting from 2 wk posttransplantation and FPP was eventually rejected 8 wk posttransplantation. Ab depletion of CD8 cells greatly improved the survival of FPP and reduced cell infiltration at the graft site. This suggests that CD8 cells can mediate the rejection of porcine xenografts in the absence of CD4 cells. This CD8-mediated rejection of FPP is independent of their perforin-mediated lytic function, as graft survival was not affected in mice deficient in perforin. The prodn. of IFN-.gamma. and IL-5 by the graft infiltrates indicates that CD8 cells may act through cytokine-mediated mechanisms. Remarkably, in the absence of CD4 cells, lymphocyte infiltration at the graft site was absent in mice transgenic for **CTLA4Ig** such that the **islet** grafts flourished beyond 24 wk. In contrast, rejection was little affected by CD40 ligand deficiency. Therefore, we show that CD8 cells are activated to mediate FPP rejection independent of perforin and that this CD4-independent activation of CD8 cells critically depends on B7/CD28 costimulation.

REFERENCE COUNT: 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 3 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:513887 HCAPLUS
DOCUMENT NUMBER: 136:182116
TITLE: Immunotherapy with nondepleting anti-CD4 monoclonal antibodies but not CD28 antagonists protects islet graft in spontaneously diabetic NOD mice from autoimmune destruction and allogeneic and xenogeneic graft rejection
AUTHOR(S): Guo, Zhiguang; Wu, Tao; Kirchhof, Nicole; Mital, Deepak; Williams, James W.; Azuma, Miyuki; Sutherland, David E. R.; Hering, Bernhard J.
CORPORATE SOURCE: Diabetes Institute for Immunology and Transplantation and Department of Surgery, University of Minnesota, Minneapolis, MN, 55455, USA
SOURCE: Transplantation (2001), 71(11), 1656-1665
CODEN: TRPLAU; ISSN: 0041-1337
PUBLISHER: Lippincott Williams & Wilkins
DOCUMENT TYPE: Journal
LANGUAGE: English
AB T-cell activation and the subsequent induction of effector functions require not only the recognition of antigen peptides bound to MHC mols. by T-cell receptor (TCR) for antigen but also a costimulatory signal provided by antigen presenting cells. CD4 T-cell activation and function require the CD4 mol. as a coreceptor of TCR. The CD28/B7 pathway is a major costimulatory signal for T-cell activation and differentiation. The effect of targeting CD4 by nondepleting anti-CD4 monoclonal antibodies (mAbs) vs. blocking CD28/B7 by **CTLA4Ig**, anti-CD80 mAbs, and anti-CD86 mAbs on the prevention of recurrence of autoimmune diabetes after MHC-matched nonobese diabetes-resistant (NOR) **islet** transplantation in nonobese diabetic (NOD) mice were compared. Whether nondepleting anti-CD4 mAbs prolong allogeneic **islet** graft survival and xenogeneic pig **islet** graft survival in diabetic NOD mice were studied. Furthermore, the effect of nondepleting anti-CD4 mAbs combined with **CTLA4Ig** on allogeneic **islet** graft survival in NOD mice was investigated. Recurrence of autoimmune diabetes can be prevented by nondepleting anti-CD4 mAbs. Blocking the CD28/B7 costimulatory pathway by **CTLA4Ig** or by anti-CD80 mAbs and anti-CD86 mAbs cannot prevent recurrence of autoimmune diabetes after **islet** transplantation. Short-term treatment with nondepleting anti-CD4 mAbs significantly prolongs allogeneic **islet** graft survival and xenogeneic pig **islet** graft survival in diabetic NOD mice. But nondepleting anti-CD4 mAbs combined with **CTLA4Ig** decreased allogeneic **islet** graft survival. Nondepleting anti-CD4 mAbs but not CD28 antagonists protect **islet** grafts in diabetic NOD mice from autoimmune destruction and allogeneic and xenogeneic graft rejection. The efficacy of nondepleting anti-CD4 mAbs is compromised when it combines with **CTLA4Ig**.
REFERENCE COUNT: 63 THERE ARE 63 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 4 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:209116 HCAPLUS
DOCUMENT NUMBER: 135:330410
TITLE: Mechanism of adenoviral-mediated CTLA4-Ig gene-induced pancreatic allograft tolerance in rats
AUTHOR(S): Liu, C.; Deng, S.; Jiang, K.; Gelman, A.; Shaked, A.; Brayman, K. L.
CORPORATE SOURCE: Department of Surgery, University of Pennsylvania, Philadelphia, PA, USA

Searched by M. Smith

SOURCE: Transplantation Proceedings (2001), 33(1-2), 134
CODEN: TRPPA8; ISSN: 0041-1345
PUBLISHER: Elsevier Science Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The study aims to evaluate whether radiosensitive antigen-presenting cells (APCs) in the donor pancreas may play an important role in induction of tolerance in the rat pancreas transplantation model. Cells were eliminated in the graft by lethal irradn. of the donor pancreas prior to procurement. It was also assessed whether tolerance could be abrogated following transplantation of irradiated pancreatic grafts transduced with AdCTLA-4Ig. Destruction of islets was found in the irradiated control or transduced allografts in addn. to fibrosis within the grafts. Irradn. and, probably, elimination of APCs from the pancreas allograft led to considerable redn. in the survival time of the AdCTLA4Ig-transduced grafts. This indicated that absence of APCs may override the immunosuppressive effect of the CTLA4-Ig transgene in the rodent pancreas transplant model.

REFERENCE COUNT: 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 5 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:554472 HCAPLUS
DOCUMENT NUMBER: 134:216953
TITLE: Efficacy of FK506, leflunomide, anti-CD4, and **CTLA4IG** treatments in rat to mouse **pancreas** xenograft transplantation
AUTHOR(S): Yin, D. P.; Sankary, H. N.; Shen, J.; Ma, L. L.; Blinder, L.; Foster, P.; Williams, J. W.; Chong, A.
CORPORATE SOURCE: Division of Transplantation, Department of General Surgery, Rush-Presbyterian St Luke's Medical Center, Chicago, IL, USA
SOURCE: Transplantation Proceedings (2000), 32(5), 1003-1004
CODEN: TRPPA8; ISSN: 0041-1345
PUBLISHER: Elsevier Science Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A study was conducted to assess the effect of FK506, leflunomide, anti-CD4, anti-CD8, and muCTLA4Ig in prolonging survival of **pancreas** xenografts in Lewis rat to streptozotocin-induced diabetic C57BL/6 mice. Results show that FK506 treatment induced long-term survival of xenogeneic **pancreas**. The role of T cells in rejection of xenogeneic **pancreas** was also confirmed by the ability of anti-CD4 and of **CTLA4Ig** to significantly prolong survival of Lewis **pancreas** grafts. However, anti-CD8 alone can only slightly prolong survival of **pancreas** xenografts.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 6 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:481472 HCAPLUS
DOCUMENT NUMBER: 134:16405
TITLE: Role of **CTLA4Ig** gene in transplantation of **pancreatic islets** in rats
AUTHOR(S): Jing, Shi-Long; Gu, Hong-Guang; Wang, Ya-Xu; Liu, Bao-Hua; Wen, Ya-Yuan; Wang, Ren-Jun; Wang, Dong; He, Pei-Sheng
CORPORATE SOURCE: Daping Hospital, Third Military Medical University, Chungking, 400042, Peop. Rep. China

SOURCE: Di-San Junyi Daxue Xuebao (2000), 22(5), 433-435
CODEN: DYXUE8; ISSN: 1000-5404
PUBLISHER: Di-San Junyi Daxue
DOCUMENT TYPE: Journal
LANGUAGE: Chinese

AB Objective To study the expression of **CTLA4Ig** gene and the effect of its product on the prolongation of the survival of **pancreatic islet** allograft in diabetic rats. Methods The cells of the **islets** and muscles of the **pancreas** were transfected with **CTLA4Ig** cDNA which was packaged with libofectin vector. The expression of **CTLA4Ig** gene was obsd. after the transplantation of **pancreatic islets** to see the effect on the prolongation of the survival of the allografts and of the diabetic rats. Results The expression of **CTLA4Ig** in serum was pos. in 2 out of 10 rats in group A with concns. of 14 ng/mL and 31ng/mL resp. 7 days after the transplantation. The av. duration of normal blood glucose level after the transplantation was 14.8 12.3 days in group A and 3.6 5.1 days in group B($P < 0.05$). The av. survival time of the transplanted rats was 24.0 10.8 days (max. of 45 days) in group A and 10.8+4.8 days(max. of 21 days) in group B ($P < 0.01$). Conclusion **CTLA4Ig** gene was expressed in the cells of **islets** and muscles of the **pancreas** in the recipient rats and prolonged the survival of the **pancreatic islet** allograft as well as the recipient rats significantly.

L6 ANSWER 7 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:414578 HCAPLUS
DOCUMENT NUMBER: 134:70182
TITLE: Protective effect of **CTLA4Ig** secreted by transgenic fetal **pancreas** allografts
AUTHOR(S): Sutherland, Robyn M.; Brady, Jamie L.; Georgiou, Harry M.; Thomas, Helen E.; Lew, Andrew M.
CORPORATE SOURCE: Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, Parkville, 3050, Australia
SOURCE: Transplantation (2000), 69(9), 1806-1812
CODEN: TRPLAU; ISSN: 0041-1337
PUBLISHER: Lippincott Williams & Wilkins
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Background. **Pancreas** allotransplantation offers a cure for insulin-dependent diabetes mellitus. Systemic immunosuppression used to prevent immune destruction of the graft has side-effects, including increased susceptibility to infection and neoplasia. These unwanted effects may be limited by engineering the graft to secrete immunomodulatory mols., to achieve local immunosuppression. Several studies have shown that transient local **CTLA4Ig** results in partial protection of allogeneic grafts. Our intent has been to det. whether sustained secretion of transgenic **CTLA4Ig** from **pancreatic islets** is able to protect against allograft rejection. Methods and Results. Mouse CTLA4 (test=**CTLA4Ig**) or CD5 leader sequence (control=CD5LIg) was fused to the Fc of mouse IgG2c, and expressed transgenically under the control of the rat insulin promoter in C57BL/6 mice carrying the bm1 mutation of H-2Kb (B6.C-H-2bm1). This resulted in expression in **pancreatic islets**. We used ELISA quantification of transgene products secreted into the supernatants of cultured fetal **pancreata** to select high (CTLA4Ighi) and low (CTLA4Iglo) expresser transgenic mice. Cultured fetal **pancreata** were transplanted under the kidney capsule of wholly allogeneic CBA recipient mice. CTLA4Ighi but not CTLA4Iglo expresser grafts showed

enhanced survival compared with control CD5LIg grafts at 6 wk posttransplant, provided the recipient mice were transiently depleted of CD4 T cells (by a single low-dose injection of GK1.5) before transplantation. Conclusions. Sustained local secretion of **CTLA4Ig** from transgenic grafts in combination with transient systemic CD4 T-cell depletion can enhance allograft acceptance.
REFERENCE COUNT: 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 8 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:264347 HCAPLUS
DOCUMENT NUMBER: 133:295072
TITLE: Additive efficacy of CTLA4Ig and OX40Ig secreted by genetically modified grafts
AUTHOR(S): Brady, Jamie L.; Lew, Andrew M.
CORPORATE SOURCE: Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, Parkville, 3050, Australia
SOURCE: Transplantation (2000), 69(5), 724-730
CODEN: TRPLAU; ISSN: 0041-1337
PUBLISHER: Lippincott Williams & Wilkins
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Background. The use of systemic immunosuppressive drugs have been paramount in the success in transplantation, but there are serious deleterious effects. Genetic modification of grafts to secrete immunomodulators locally may be a way to reduce the need for systemic immunosuppression. Methods and Results. An insulinoma cell line, NIT, having the nonobese diabetic (NOD) genotype but also expressing the SV40 large T Ag, was transfected with CTLA4Ig or OX40Ig in an attempt to block signals in the costimulatory/adhesion pathways. The extracellular domains of these mols. have been fused to the Fc of IgG2c derived from the NOD mouse strain. This resulted in secreted and dimerized proteins. SV40 T Ag is potent at inducing graft rejection. Test and control transfectants were transplanted s.c. into young NOD mice to det. whether secretion of CTLA4Ig and OX40Ig would promote survival of the insulinoma graft. In immunodeficient mice, cell growth was similar for all transfectants. However, in immunocompetent NOD mice, the survival/growth of test grafts was significantly better than that of controls. By combining test transfectants, we found that graft survival was enhanced in an additive and significant fashion. In vitro, there was a significant redn. in immune responses-compared with control-when purified fusion proteins were added to mixed leukocyte reaction cultures. Conclusions. We conclude that blockade of individual costimulatory/adhesion signals by graft manipulation can contribute to transplantation success and that blockade of combinations of signals in these pathways enhances this success. Successful immunomodulation by the graft itself can be achieved.
REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 9 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:26501 HCAPLUS
DOCUMENT NUMBER: 132:264056
TITLE: The effect of **CTLA4Ig** gene on **pancreatic islet** allograft for diabetic rat
AUTHOR(S): Jin, Shilong; Gu, Hongguang; Wang, Yaxiu; Liu, Baohua; Wang, Daike; Wen, Yayuan; Wang, Renyun; Wang, Dong; Zhou, Yueqing; Liu, Ping
CORPORATE SOURCE: Dep. General Surgery, Third Military Med. Univ.,

SOURCE: Chungking, 400042, Peop. Rep. China
Zhongguo Puwai Jichu Yu Linchuang Zazhi (1999), 6(6), 328-330
CODEN: ZJLZFX; ISSN: 1007-9424
PUBLISHER: Zhongguo Puwai Jichu Yu Linchuang Zazhi Bianji Weiyuanhui
DOCUMENT TYPE: Journal
LANGUAGE: Chinese

AB To study the expression of **CTLA4Ig** gene in diabetic rats and the effect of **CTLA4Ig** on long-term survival of the **pancreatic islet**. The rat **pancreatic islet** cell and muscle cell transfected with the cDNA for **CTLA4Ig** packaged with lipofectin vector. We examd. the expression level of **CTLA4Ig** gene, T lymphocyte reaction and obsd. the expression of **CTLA4Ig** cDNA in diabetic rat and the action of **CTLA4Ig** in long-term survival of **pancreatic islet** transplanted and the transplanted rats. Results: For the T lymphocyte reaction from peripheral i.v. blood at seventh day after surgery, the difference between two groups was significant ($P < 0.05$). Only 2 out of 10 recipients of the expt. group (A) at the seventh day after **pancreatic islet** allograft had any detectable levels of **CTLA4Ig**, and their concn. was 14 ng/mL, and 31 ng/mL. The av. time of maintaining blood glucose in normal levels of the group A after **pancreatic islets** graft, 14.8 .+-. 12.3 days, was significantly longer than 3.6 .+-. 5.1 days of the control group (B) ($P < 0.05$). The av. survival time of the group A, 24.0 .+-. 10.8 days (the longest time was 45 days), was significantly longer than 11.8 .+-. 4.8 days (the longest time was 21 days) of the group B ($P < 0.01$). Conclusions: The muscle cells and **pancreatic islets** of the recipient rat were transfected with **CTLA4Ig** cDNA packaged with lipofectin, and **CTLA4Ig** cDNA was expressed in recipient tissue, its expressed product **CTLA4Ig** make **pancreatic islets** transplanted and recipient rat survive longer significantly.

L6 ANSWER 10 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:523056 HCAPLUS
DOCUMENT NUMBER: 131:267615
TITLE: Genetic engineering of dendritic cells to express immunosuppressive molecules (viral IL-10, TGF-.beta., and CTLA4Ig)
AUTHOR(S): Lu, Lina; Lee, Wei-Chen; Takayama, Takuya; Qian, Shiguang; Gambotto, Andrea; Robbins, Paul D.; Thomson, Angus W.
CORPORATE SOURCE: Thomas E. Starzl Transplantation Institute and Department of Surgery, University of Pittsburgh, PA, USA
SOURCE: Journal of Leukocyte Biology (1999), 66(2), 293-296
CODEN: JLBIE7; ISSN: 0741-5400
PUBLISHER: Federation of American Societies for Experimental Biology
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

AB A review with 31 refs. There is growing evidence that, in addn. to their role as initiators of immune responses, dendritic cells (DC) can exhibit tolerogenic properties. Immature DC deficient in cell surface costimulatory/accessory mols. can prolong organ and **pancreatic islet** allograft survival, whereas in vitro manipulation of DC by exposure to a variety of factors (e.g., viral interleukin-10;

CTLA4Ig) can confer tolerogenic properties on these cells. Genetic engineering of DC to express immunosuppressive mols. is, in theory, an attractive approach to the therapy of allograft rejection and possibly, autoimmune disorders.
REFERENCE COUNT: 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 11 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:477238 HCAPLUS
DOCUMENT NUMBER: 132:22109
TITLE: Prolonged xenograft survival of **islets** infected with small doses of adenovirus expressing **CTLA4Ig**
AUTHOR(S): Feng, Sandy; Quickel, Robert R.; Hollister-Lock, Jennifer; McLeod, Matthew; Bonner-Weir, Susan; Mulligan, Richard C.; Weir, Gordon C.
CORPORATE SOURCE: Department of Genetics, Harvard Medical School and Howard Hughes Medical Institute, The Children's Hospital, Boston, MA, 02115, USA
SOURCE: Transplantation (1999), 67(12), 1607-1613
CODEN: TRPLAU; ISSN: 0041-1337
PUBLISHER: Lippincott Williams & Wilkins
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Systemic administration of the inhibitor of costimulation, **CTLA4Ig**, has been shown to prolong **islet** graft survival. The purpose of this study was to compare local and systemic expression of murine **CTLA4Ig** in transplants of rat **islets** into mice. Murine **CTLA4Ig** was made by joining two polymerase chain reaction products, the extracellular portion of CTLA4 and the Fc portion of IgG2a. Recombinant adenovirus expressing **CTLA4Ig** (AdCTLA4Ig) was generated using the strategy of Cre-lox recombination. Isolated rat **islets** infected with AdCTLA4Ig at multiplicities of infection (MOIs) ranging from 0.1 to 10 were transplanted into streptozocin diabetic male B6AF1 mice. Control **islets** were mock infected or infected with AdLacZ or AdsIg, a recombinant adenovirus expressing only the Fc portion of IgG2a. Also, AdCTLA4Ig and control viruses were injected i.m. into mouse transplant recipients at the time of **islet** transplantation to provide **CTLA4Ig** systemically. Control **islets** transplanted into diabetic mice were rejected in 13-17 days. **Islets** infected with AdCTLA4Ig had dose-dependent prolongation of graft survival. Prolonged survival was even found with very low MOIs of 0.1 and 0.5, with survivals of 24 and 25 days, resp. Survival with an MOI of 10 was 39 days. With i.m. injection, no prolongation was found at the lowest relative MOIs of 0.2 and 1, but there was dose-dependent prolongation of graft survival with larger doses. At the highest relative MOI of 400, survival was prolonged to 58.+-.10 days. Rat **islets** infected with AdCTLA4Ig transplanted into mice had prolonged graft survival. Prolonged survival with MOIs as low as 0.1 and 0.5 indicates that only a minority of **islet** cells need to express **CTLA4Ig** to exert an effect. Moreover, the results suggest that the improved **islet** graft survival is due to a local influence of **CTLA4Ig**.
REFERENCE COUNT: 43 THERE ARE 43 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 12 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:185728 HCAPLUS
DOCUMENT NUMBER: 131:17960

TITLE: Prevention of T-cell activation by rhCTLA4-Ig and anti-CD40L monoclonal antibody results in indefinite islet allograft survival

AUTHOR(S): Rastellini, C.; Salam, A.; Kuddus, R.; Aitouche, A.; Subbotin, V.; Braun, M.; Leach, R.; Peach, R.; Fung, J. J.; Starzl, T. E.; Rao, A. S.

CORPORATE SOURCE: Section of Cellular Transplantation, Thomas E. Starzl Transplantation, University of Pittsburgh Medical Center, Pittsburgh, PA, 15213, USA

SOURCE: Transplantation Proceedings (1999), 31(1/2), 1242-1243
CODEN: TRPPA8; ISSN: 0041-1345

PUBLISHER: Elsevier Science Inc.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB It has been reported that the use of either rhCTLA4-Ig or anti-CD40L MAb alone resulted in prolongation but not indefinite survival of islet allografts. The authors hypothesized that combined transient perioperative blockade of both costimulatory pathways would result in indefinite islet allograft survival, thus allowing maintenance of euglycemia in a diabetic recipient without the need for exogenous insulin. Indefinite islet allograft survival was witnessed in recipients treated perioperatively with both rhCTLA4-Ig and anti-CD40L MAb. Clin. translation of this strategy would greatly improve survival of islets allotransplanted into insulin-dependent type I diabetes with long-term freedom from exogenous insulin.

REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 13 OF 22 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1999:158562 HCAPLUS

DOCUMENT NUMBER: 130:324287

TITLE: Prevention of autoimmune recurrence and rejection by adenovirus-mediated **CTLA4Ig** gene transfer to the **pancreatic** graft in BB rat

AUTHOR(S): Uchikoshi, Fumihiro; Yang, Zan-Dong; Rostami, Susan; Yokoi, Yoshihiro; Capocci, Pam; Barker, Clyde F.; Naji, Ali

CORPORATE SOURCE: Department of Surgery, University of Pennsylvania Medical Center, Philadelphia, PA, 19104, USA

SOURCE: Diabetes (1999), 48(3), 652-657
CODEN: DIAEAZ; ISSN: 0012-1797

PUBLISHER: American Diabetes Association

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Type 1 diabetes is the result of a selective destruction of **pancreatic islets** by autoreactive T-cells. Therefore, in the context of **islet** or **pancreas** transplantation, newly transplanted .beta.-cells are threatened by both recurrent autoimmune and alloimmune responses in recipients with type 1 diabetes. In the present study, using spontaneously diabetic BB rats, we demonstrate that whereas isolated **islets** are susceptible to autoimmune recurrence and rejection, **pancreaticoduodenal** grafts are resistant to these biol. processes. This resistance is mediated by lymphohematopoietic cells transplanted with the graft, since inactivation of these passenger cells by irradn. uniformly rendered the **pancreaticoduodenal** grafts susceptible to recurrent autoimmunity. We further studied the impact of local immunomodulation on autoimmune recurrence and rejection by ex vivo adenovirus-mediated **CTLA4Ig** gene transfer to **pancreaticoduodenal** grafts. Syngeneic DR-BB

pancreaticoduodenal grafts transduced with AdmCTLA4Ig were rescued from recurrent autoimmunity. In fully histoincompatible LEW.fwdarw.BB transplants, in which rejection and recurrence should be able to act synergistically, AdmCTLA4Ig transduced LEW-**pancreaticoduodenal** allografts enjoyed markedly prolonged survival in diabetic BB recipients. In situ reverse transcription-polymerase chain reaction revealed that transferred **CTLA4Ig** gene was strongly expressed in both endocrine and exocrine tissues on day 3. These results indicate the potential utility of local CD28-B7 costimulatory blockade for prevention of alloimmune and autoimmune destruction of **pancreatic** grafts in type 1 diabetic hosts.

REFERENCE COUNT: 21 THERE ARE 21 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 14 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:544484 HCAPLUS
DOCUMENT NUMBER: 129:301276
TITLE: Immunomodulation of islet allografts with genetically modified muscle cells
AUTHOR(S): Yu, Ming; Chahine, A. Alfred; Stoeckert, Christian; Lau, Henry T.
CORPORATE SOURCE: The Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, PA, USA
SOURCE: Local Immunosuppression of Organ Transplants (1996), 181-192. Editor(s): Gruber, Scott A. Landes: Austin, Tex.
CODEN: 66OCAO
DOCUMENT TYPE: Conference; General Review
LANGUAGE: English
AB A review and discussion with 39 refs. It is now possible to block selected and successive steps in the rejection cascade. The authors propose that it is possible to deliver recombinant immunosuppressive mols. by genetically engineering recipient type muscle cells such that they become carrier cells for the prodn. of recombinant protein. This promotes allograft survival and allows one to further immune activation. One example is the use of syngeneic **CTLA4Ig**-secreting muscle cells which prolong survival of **islet** cell allografts.

L6 ANSWER 15 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:776907 HCAPLUS
DOCUMENT NUMBER: 128:60679
TITLE: Immunosuppressive effects of human **CTLA4Ig** in a non-human primate model of allogeneic **pancreatic islet** transplantation
AUTHOR(S): Levisetti, Matteo G.; Padrid, Philip A.; Szot, Gregory L.; Mittal, Naveen; Meehan, Shane M.; Wardrip, Craig L.; Gray, Gary S.; Bruce, David S.; Thistlethwaite, J. R., Jr.; Bluestone, Jeffrey A.
CORPORATE SOURCE: Department of Surgery, University of Chicago, Chicago, IL, 60637, USA
SOURCE: Journal of Immunology (1997), 159(11), 5187-5191
CODEN: JOIMA3; ISSN: 0022-1767
PUBLISHER: American Association of Immunologists
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Ag-specific T cell activation requires a CD28-mediated costimulatory interaction. This observation has suggested novel approaches to suppress donor-specific immunity, including the use of sol. CD28 antagonists, such as **CTLA4Ig**, which suppresses transplant rejection in small

animal models. In this study, **CTLA4Ig** therapy was examd. in a non-human primate model of allogeneic **pancreatic islet** transplantation. Two of five **CTLA4Ig**-treated monkeys showed prolonged graft survival, which correlated with donor-specific hyporesponsiveness in vitro. Humoral responses to the transplanted tissue were suppressed in all treated animals. These results suggest that **CTLA4Ig** is effective in suppressing both humoral and cellular immune responses in a non-human primate model of allogeneic transplantation.

L6 ANSWER 16 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:536552 HCAPLUS
DOCUMENT NUMBER: 127:204372
TITLE: **CTLA4Ig** attenuates accelerated rejection (presensitization) in the mouse **islet** allograft model
AUTHOR(S): Roy-Chaudhury, Prabir; Nickerson, Peter W.; Manfro, Roberto C.; Zheng, Xin Xiao; Steiger, Jurg; Li, Yong Sheng; Strom, Terry B.
CORPORATE SOURCE: Department of Medicine, Division of Immunology, Beth Israel Hospital, Harvard Medical School, Boston, MA, 02215, USA
SOURCE: Transplantation (1997), 64(1), 172-175
CODEN: TRPLAU; ISSN: 0041-1337
PUBLISHER: Williams & Wilkins
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Sensitization to donor antigens is a problem of growing magnitude in clin. transplantation. At a mol. level, this is due to the interaction between antigen bearing antigen-presenting cells and recipient T cells and involves both antigen presentation and co-stimulation. Allogeneic **islet** transplantation was performed using DBA/2J donors and B6AF1 recipients. Four weeks before transplantation, recipient animals were given donor-specific transfusion (DST) alone, DST + **CTLA4Ig**, DST + control IgG, or no treatment. Graft loss was defined as a blood glucose >300 mg/100 mL. Administration of DST + control IgG 4 wk before transplantation resulted in accelerated rejection due to presensitization (median survival time of 8 days, compared with 14.5 days for the no-treatment group). Animals treated with **CTLA4Ig** in combination with DST had a median survival time of 12 days, compared with 8 days for DST + IgG. **CTLA4Ig** attenuates the tempo of accelerated rejection in this **islet** allograft model of presensitization, but does not prolong allograft survival as compared with no treatment.

L6 ANSWER 17 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:476344 HCAPLUS
DOCUMENT NUMBER: 127:204415
TITLE: The role of donor and recipient B7-1 (CD80) in allograft rejection
AUTHOR(S): Zheng, Xiu Xiao; Sayegh, Mohamed H.; Zheng, Xiang-Guang; Li, Yongsheng; Linsley, Peter S.; Peach, Robert; Borriello, Frank; Strom, Terry B.; Sharpe, Arlene H.; Turka, Laurence A.
CORPORATE SOURCE: Dep. Medicine, Beth-Israel Deacones Medical Center, Boston, MA, 02215, USA
SOURCE: Journal of Immunology (1997), 159(3), 1169-1173
CODEN: JOIMA3; ISSN: 0022-1767
PUBLISHER: American Association of Immunologists

DOCUMENT TYPE: Journal
LANGUAGE: English

AB Blockade of CD28-mediated T cell costimulatory signals produces effective immunosuppression of a variety of T cell-dependent in vivo immune responses, including autoimmune disorders and transplant rejection. The sol. fusion protein **CTLA4Ig**, which competitively blocks CD28 ligands B7-1 and B7-2, can prevent allograft and xenograft rejection and in some circumstances induce transplantation tolerance. To det. the relative roles of B7-1 and B7-2 in graft rejection, the authors performed **islet** and cardiac allografts with normal and B7-1-/- mice in conjunction with selective blocking reagents. They found that the absence of B7-1 on donor or recipient tissues leads to a slight prolongation of **islet** allograft survival, but has minimal or no effect on cardiac allograft survival. Allograft function is further prolonged in the **islet** model when both donor and recipient lack B7-1, although cardiac allograft survival is not prolonged. In the cardiac model, treatment with **CTLA4Ig** induces long term survival in B7-1-/- recipients regardless of donor status. In contrast, anti-B7-2 mAb leads to indefinite allograft survival only when the recipient and donor both lack B7-1, indicating that even in the absence of available B7-2, B7-1 mols. on the donor or recipient cells alone are sufficient to induce graft rejection. Also, B7-1 and B7-2 are the only CD28 ligands relevant to cardiac allograft rejection in mice.

L6 ANSWER 18 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1996:164054 HCAPLUS
DOCUMENT NUMBER: 124:229983
TITLE: CTLA4 molecules and IL-4-binding molecules and uses thereof
INVENTOR(S): Linsley, Peter S.; Ledbetter, Jeffrey A.; Damle, Nitin; Brady, William; Wallace, Philip M.; Peach, Robert J.
PATENT ASSIGNEE(S): Bristol-Myers Squibb Co., USA
SOURCE: Can. Pat. Appl., 124 pp.
CODEN: CPXXEB
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 8
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
CA 2146895	AA	19951016	CA 1995-2146895	19950412
US 6090914	A	20000718	US 1994-228208	19940415

PRIORITY APPLN. INFO.:
US 1994-228208 A 19940415
US 1991-723617 B2 19910627
US 1993-8898 A2 19930122

AB The invention identifies the CTLA4 receptor as a ligand for the B7 antigen. The complete amino acid sequence encoding human CTLA4 receptor gene is provided. Methods are provided for expressing CTLA4 as an Ig fusion protein, for prepg. hybrid CTLA4/CD28 fusion proteins, and for using the sol. fusion proteins, fragments and derivs. thereof, including monoclonal antibodies reactive with B7 and CTLA4, to regulate T cell interactions and immune responses mediated by such interactions. In example, B7-Ig.C.gamma.1, CD28-Ig.C.gamma.1 and CTLA4-Ig.C.gamma.1 fusion proteins were prepd., purified., characterized, and used for inhibiting immune response in vitro, Ig. secretion, and for preventing rejection of **islet** transplant in mice. Also mutant **CTLA4Ig** and CD28Ig fusion proteins and CTLA4/CD28Ig hybrid fusion proteins were prepd.

and tested for their binding to CTLA4 and CD28 monoclonal antibodies.

L6 ANSWER 19 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1995:627461 HCAPLUS
DOCUMENT NUMBER: 123:110034
TITLE: Immunomodulation of **pancreatic islet** allografts in mice with **CTLA4Ig** secreting muscle cells
AUTHOR(S): Chahine, A. Alfred; Yu, Ming; McKernan, Melissa M.; Stoeckert, Christian; Lau, Henry T.
CORPORATE SOURCE: Children's Hospital of Philadelphia, University of Pennsylvania, Philadelphia, 19104, USA
SOURCE: Transplantation (1995), 59(9), 1313-18
CODEN: TRPLAU; ISSN: 0041-1337
DOCUMENT TYPE: Journal
LANGUAGE: English
AB In an effort to create a model of in vivo prodn. of immunosuppressants, the authors have transfected C2C12 muscle cells (H-2k) with the cDNA for **CTLA4Ig**, a fusion protein that prevents the activation of T cells by blocking the costimulatory signal transduced by the T cell receptors CD28 and CTLA4. **CTLA4Ig**-secreting clones were cotransplanted with **islets** as composite grafts in the renal subcapsular space of diabetic mice. When the myoblasts were syngeneic to C3H/HeJ hosts (H-2k), there was a significant prolongation of survival of allogeneic C57BL/6J (H-2b) **islets** from a mean 11.0 days to 31.7 days. When the graft was completely allogeneic (H-2k myoblasts and **islets** into H-2b recipients), there was no benefit in survival. A transient blockade of LFA-1 with the mAb M17 was synergistic in this combination: 8 out of 12 C57BL/6J recipients achieved long-term acceptance. Systemic **CTLA4Ig** levels were detected up to 60 days after transplantation. In conclusion, the authors have shown that C2C12 muscle cells can be genetically engineered to secrete functional **CTLA4Ig** and that they can be used as a gene reservoir for the continuous in vivo prodn. of **CTLA4Ig** to modulate the survival of **islet** cell allografts.

L6 ANSWER 20 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1995:381690 HCAPLUS
DOCUMENT NUMBER: 122:158062
TITLE: Differential effects of anti-B7-1 and anti-B7-2 monoclonal antibody treatment on the development of diabetes in the nonobese diabetic mouse
AUTHOR(S): Lenschow, Deborah J.; Ho, Stephen C.; Sattar, Husain; Rhee, Lesley; Gray, Gary; Nabavi, Nasrin; Herold, Kevan C.; Bluestone, Jeffrey A.
CORPORATE SOURCE: Ben May Inst. Committe Immunol., Univ. Chicago, Chicago, IL, 60637, USA
SOURCE: Journal of Experimental Medicine (1995), 181(3), 1145-55
CODEN: JEMEAV; ISSN: 0022-1007
PUBLISHER: Rockefeller University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Insulin-dependent diabetes mellitus (IDDM) is thought to be an immunol.-mediated disease resulting in the complete destruction of the insulin-producing **islets** of Langerhans. It has become increasingly clear that autoreactive T cells play a major role in the development and progression of this disease. In this study, the authors examd. the role of the CD28/B7 costimulation pathway in the development

and progression of autoimmune diabetes in the nonobese diabetic (NOD) mouse model. Female NOD mice treated at the onset of insulitis (2-4 wk of age) with **CTLA4Ig** (a sol. CD28 antagonist) or a monoclonal antibody (mAb) specific for B7-2 (a CD28 ligand) did not develop diabetes. However, neither of these treatments altered the disease process when administered late, at >10 wk of age. Histol. examn. of **islets** from the various treatment groups showed that while **CTLA4Ig** and anti-B7-2 mAb treatment blocked the development of diabetes, these reagents had little effect on the development or severity of insulitis. Together these results suggest that blockade of costimulatory signals by **CTLA4Ig** or anti-B7-2 acts early in disease development, after insulitis but before the onset of frank diabetes. NOD mice were also treated with mAbs to another CD28 ligand, B7-1. In contrast to the previous results, the anti-B7-1 treatment significantly accelerated the development of disease in female mice and, most interestingly, induced diabetes in normally resistant male mice. A combination of anti-B7-1 and anti-B7-2 mAbs also resulted in an accelerated onset of diabetes, similar to that obsd. with anti-B7-1 mAb treatment along, suggesting that anti-B7-1 mAb's effect was dominant. Furthermore, treatment with anti-B7-1 mAbs resulted in a more rapid and severe infiltrate. Finally, T cells isolated from the **pancreases** of these anti-B7-1-treated animals exhibited a more activated phenotype than T cells isolated from any of the other treatment groups. These studies demonstrate that costimulatory signals play an important role in the autoimmune process, and that different members of the B7 family have distinct regulatory functions during the development of autoimmune diabetes.

L6 ANSWER 21 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1992:548893 HCAPLUS
DOCUMENT NUMBER: 117:148893
TITLE: Long-term survival of xenogeneic **pancreatic islet** grafts induced by **CTLA4Ig**
AUTHOR(S): Lenschow, Deborah J.; Zeng, Yijun; Thistlethwaite, J. Richard; Montag, Anthony; Brady, William; Gibson, Marylou G.; Linsley, Peter S.; Bluestone, Jeffrey A.
CORPORATE SOURCE: Ben May Inst., Univ. Chicago, Chicago, IL, 60637, USA
SOURCE: Science (Washington, DC, United States) (1992), 257(5071), 789-92
CODEN: SCIEAS; ISSN: 0036-8075
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Antigen-specific T cell activation depends on T cell receptor-ligand interaction and costimulatory signals generated when accessory mols. bind to their ligands, such as CD28 to the B7 (also called BB1) mol. A sol. fusion protein of human CTLA-4 (a protein homologous to CD28) and the IgG1 Fc region (**CTLA4Ig**) binds to human and murine B7 with high avidity and blocks T cell activation in vitro. **CTLA4Ig** therapy blocked human **pancreatic islet** rejection in mice by directly affecting T cell recognition of B7+ antigen-presenting cells. In addn., **CTLA4Ig** induced long-term, donor-specific tolerance, which may have applications to human organ transplantation.

L6 ANSWER 22 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1987:508944 HCAPLUS
DOCUMENT NUMBER: 107:108944
TITLE: Morphology of the chronic toxicity of busulfan on the islets of Langerhans in the rat
AUTHOR(S): Kaduk, Bernhard; Husslein, Eva Maria; Siegfried, Alena
CORPORATE SOURCE: Inst. Pathol., Univ. Erlangen-Nuremberg, Erlangen,

8520, Fed. Rep. Ger.
SOURCE: Hepato-Gastroenterology (1987), 34(3), 108-12
CODEN: HEGAD4; ISSN: 0172-6390
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Male Wistar rats were fed busulfan dissolved in fat milk at a daily dose of 0.35, 0.89, 1.20 mg/kg for a period of 4, 8, and 12 wk. Examn. by light microscopy, including immunohistochem., and by electron microscopy revealed changes in the pancreatic islets which were dependent on dose and time. First, hyperemia was demonstrated, then perivascular "inbleedings" were followed by organization and islet fibrosis with centripetal redn. of the islet cells. This resulted in almost complete loss of A-cells and a redn. in the no. of B-cells. A morphol. factor anal. suggested addnl. analogies to type 1-diabetes mellitus, so that for the formal pathogenesis, islet haematoma and immunol. events have to be discussed.
IT **55-98-1**, Busulfan
RL: PRP (Properties)
(toxicity of, to **islets** of Langerhans)

HOW MANY MORE ANSWERS DO YOU WISH TO SCAN? (1):0 d
'0 D' IS NOT VALID HERE

To display more answers, enter the number of answers you would like to see. To end the display, enter "NONE", "N", "0", or "END".
HOW MANY MORE ANSWERS DO YOU WISH TO SCAN? (1):0

=> d stat que
L1 3 SEA FILE=REGISTRY (BUSULFAN/CN OR "BUSULFAN MANNITOL"/CN OR "BUSULFAN-METHOTREXATE MIXT."/CN)
L2 1 SEA FILE=REGISTRY "CTLA4 (ANTIGEN) (SWINE GENE CTLA4 PRECURSOR) "/CN
L3 1139 SEA FILE=HCAPLUS L1 OR BISULFAN?
L4 298 SEA FILE=HCAPLUS L2 OR CTLA4IG OR CTLA41G
L6 22 SEA FILE=HCAPLUS (L3 OR L4)(L)(PANCREA? OR ISLET?)
L8 17 SEA FILE=HCAPLUS (L3 OR L4)(L)(DIABET? OR GLUCOSE? OR BLOOD?(W) SUGAR?)
L9 5 SEA FILE=HCAPLUS L8 NOT L6

=> d ibib abs hitrn 19 1-5

L9 ANSWER 1 OF 5 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:321488 HCAPLUS
DOCUMENT NUMBER: 126:292436
TITLE: Method of inhibiting immune system destruction of transplanted viable cells
INVENTOR(S): Weber, Collin J.; Hagler, Mary K.; Linsley, Peter S.; Kapp, Judith A.
PATENT ASSIGNEE(S): Emory University, USA; Bristol-Myers Sqibb; Weber, Collin J.; Hagler, Mary K.; Linsley, Peter S.; Kapp, Judith A.
SOURCE: PCT Int. Appl., 113 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9711607	A1	19970403	WO 1996-US15577	19960927
W: AU, CA, JP, MX, US				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
CA 2232815	AA	19970403	CA 1996-2232815	19960927
AU 9673782	A1	19970417	AU 1996-73782	19960927
AU 721737	B2	20000713		
EP 877555	A1	19981118	EP 1996-936037	19960927
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2000500121	T2	20000111	JP 1997-513711	19960927

PRIORITY APPLN. INFO.: US 1995-4375P P 19950927
WO 1996-US15577 W 19960927

AB This invention provides a method of inhibiting a method of inhibiting viable cells transplanted into a subject from being destroyed by the subject's immune system which comprises: (a) contg. the viable cells, or tissue comprising the viable cells, prior to transplantation within a device comprising a semipermeable membrane; and (b) treating the subject with a substance which inhibits an immune system costimulation event in an amt. effective to inhibit the subject's immune system from responding to said contained cells or tissue. The substance which inhibits an immune system costimulation event is CTLA4 or **CTLA4Ig**. The semipermeable membrane device is a hollow fiber, disk, sphere or microcapsule that is impermeable to Igs. and/or lymphocytes. The viable cells for transplantation are endocrine cells, insulin-producing cells, hepatocytes, parathyroid cells, pituitary cells, neuroectodermal cell, etc. Also provided by this invention is a method of treating **diabetes** in a subject which comprises: (a) contg. viable insulin-producing cells, or tissue comprising such cells, within a device comprising a semipermeable membrane; (b) transplanting an effective amt. of such contained viable insulin-producing cells into the subject; and (c) treating the subject with an effective amt. of a substance, which inhibits an immune system costimulation event.

L9 ANSWER 2 OF 5 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1996:605562 HCAPLUS
DOCUMENT NUMBER: 125:245470
TITLE: CD28/B7 regulation of Th1 and Th2 subsets in the development of autoimmune diabetes
AUTHOR(S): Lenschow, Deborah J.; Herold, Kevan C.; Rhee, Lesley; Patel, Bina; Koons, Ann; Qin, Hui-Yu; Fuchs, Elaine; Singh, Bhagarith; Thompson, Craig B.; Bluestone, Jeffrey A.
CORPORATE SOURCE: Dep. Pathol., Ben May Inst. Cancer Res., Univ. Chicago, Chicago, IL, 60637, USA
SOURCE: Immunity (1996), 5(3), 285-293
CODEN: IUNIEH; ISSN: 1074-7613
PUBLISHER: Cell Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB CD28 ligation delivers a costimulatory signal important in T cell activation. This study demonstrates that the disruption of the CD28/B7 pathway early in the nonobese **diabetic** mouse strain, using CD28-/- and **CTLA4Ig** transgenic mice, promoted the development and progression of spontaneous autoimmune **diabetes**. Functional analyses of T cells isolated from CD28-deficient mice demonstrated that the GAD-specific T cells produced enhanced Th1-type cytokines (IL-2 and IFN.gamma.) and diminished Th2-type cytokine, IL-4. Moreover, there was a

decrease in serum levels of anti-GAD antibodies of the IgG1 isotype consistent with a profound suppression of Th2-type responses in these animals. Thus, the early differentiation of naive **diabetogenic** T cells into the Th2 subset is dependent upon CD28 signaling and this extends the understanding of this spontaneous autoimmune disease.

L9 ANSWER 3 OF 5 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1989:624934 HCAPLUS
DOCUMENT NUMBER: 111:224934
TITLE: Effect of busulfan on crystalline lens: glutathione, glutathione reductase and glucose-6-phosphate dehydrogenase
AUTHOR(S): Cherian, Mary; Rawall, U. M.
CORPORATE SOURCE: Sch. Sci., Gujarat Univ., Ahmedabad, 380 009, India
SOURCE: Indian Journal of Experimental Biology (1989), 27(10), 915-16
CODEN: IJEBA6; ISSN: 0019-5189
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Const. levels of glutathione and an increase in glutathione reductase activity are some of the earliest changes obsd. before the onset of any apparent opacity in the busulfan-treated rat lens. This suggests that busulfan is capable of evoking a response of the glutathione redox system which is adversely affected in cataractous lens. Glucose-6-phosphate dehydrogenase was decreased by busulfan in the cataractous lens, indicating an inhibitory action of the drug on hexose monophosphate shunt activity.
IT **55-98-1**, Busulfan
RL: BIOL (Biological study)
(**glucose** phosphate dehydrogenase and glutathione of eye lens response to, cataract induction in relation to)

L9 ANSWER 4 OF 5 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1971:418019 HCAPLUS
DOCUMENT NUMBER: 75:18019
TITLE: Effect of alloxan-induced diabetes on the fetal toxicity of thalidomide, carbutamide, and myleran in rats
AUTHOR(S): Ward, Roy J.; Readhead, S. M.
CORPORATE SOURCE: Pharm. Res. Lab., Reckitt and Sons Ltd., Kingston-upon-Hull, UK
SOURCE: Proceedings of the European Society for the Study of Drug Toxicity (1970), Volume Date 1969, 11(Probl. Species Difference Statist. Toxicol., Proc. Meet.), 151-66
CODEN: PSDTAP; ISSN: 0071-3090
DOCUMENT TYPE: Journal
LANGUAGE: English
AB An investigation was made of the fetal toxicity of thalidomide (known to give a low frequency of malformations in rats), carbutamide, and Myleran (busulfan), shown to be teratogenic to rats, in rats of the Sprague-Dawley strain made diabetic by injection with alloxan prior to conception. Fetal toxicity was studied in reference to litter size, resorptions, wt. of live young, external abnormalities, and skeletal abnormalities. The results gave some evidence that diabetic rats are more susceptible to thalidomide than normal animals: diabetes increases the fetal toxicity of thalidomide, while it has far less effect on the fetal toxicity of carbutamide and Myleran.
IT **55-98-1**

RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(teratogenic activity of, in **diabetes**)

L9 ANSWER 5 OF 5 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1961:19213 HCAPLUS
DOCUMENT NUMBER: 55:19213
ORIGINAL REFERENCE NO.: 55:3838a-c
TITLE: Influences of various antitumor substances upon blood oxygen consumption. Leucocyte and blood sugar level
AUTHOR(S): Morishita, Keiichi; et al.
CORPORATE SOURCE: Dental Coll., Tokyo
SOURCE: Shinyaku to Rinsho (1960), 9, 251-4
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable
AB **Bisulfan** (I), 6-mercaptopurine (II), 8-azaguanine (III), Thio-TEPA (IV), nitrogen mustard N-oxide (V), stilbestrol diphosphate (VI), sarcomycin (VII), or Carzinophilin (VIII) was administered to rabbits. III through VIII inhibited blood O consumption. The effects of V and VI were esp. effective. Conversely, I and II increased O consumption. Co-chlorophyllin (IX) opposed the inhibiting activity of III through VIII. IX in combination with I or II increased O consumption. Injections of III-VIII caused the increase of leucocyte after 3-5 hrs. Antitumor substances except VII and VIII produced a small increase of the **blood sugar** level.

=> d stat que
L1 3 SEA FILE=REGISTRY (BUSULFAN/CN OR "BUSULFAN MANNITOL"/CN OR "BUSULFAN-METHOTREXATE MIXT."/CN)
L2 1 SEA FILE=REGISTRY "CTLA4 (ANTIGEN) (SWINE GENE CTLA4 PRECURSOR) "/CN
L3 1139 SEA FILE=HCAPLUS L1 OR BISULFAN?
L4 298 SEA FILE=HCAPLUS L2 OR CTLA4IG OR CTLA41G
L5 73 SEA FILE=HCAPLUS (L3 OR L4) AND (PANCREA? OR ISLET?)
L6 22 SEA FILE=HCAPLUS (L3 OR L4)(L)(PANCREA? OR ISLET?)
L7 48 SEA FILE=HCAPLUS (L3 OR L4) AND (DIABET? OR GLUCOSE? OR BLOOD?(W)SUGAR?)
L8 17 SEA FILE=HCAPLUS (L3 OR L4)(L)(DIABET? OR GLUCOSE? OR BLOOD?(W)SUGAR?)
L9 5 SEA FILE=HCAPLUS L8 NOT L6
L10 131 SEA FILE=HCAPLUS ?CTLA4? AND (PANCRE? OR ISLET? OR DIABET?)
L11 109 SEA FILE=HCAPLUS L10 NOT (L6 OR L9)
L12 78 SEA FILE=HCAPLUS (L5 OR L7 OR L11) AND (TRANSPLAN? OR GRAFT?)
L13 59 SEA FILE=HCAPLUS L12 NOT (L6 OR L9)
L14 15 SEA FILE=HCAPLUS L13 AND CHIMER?

=> d ibib abs hitrn l14 1-15

L14 ANSWER 1 OF 15 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:905760 HCAPLUS
TITLE: Soluble CTLA-4 antigen mutant molecules for inhibiting allogeneic **islet transplant** rejection
INVENTOR(S): Larsen, Christian P.; Pearson, Thomas C.; Adams, Andrew B.
PATENT ASSIGNEE(S): Emory University, USA
SOURCE: PCT Int. Appl., 100 pp.

Searched by M. Smith

CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002094202	A2	20021128	WO 2002-US16708	20020523

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2001-293402P P 20010523

AB The present invention is a method of inhibiting **islet** cell **transplant** rejection particular, to treat **diabetes**, such as type-1 and type-2 **diabetes**, by administering to a subject an effective amt. of a sol. **CTLA4** mutant mol. The sol. **CTLA4** mutant mol. is L104EA29YIg, L104EIg, L104EA29LIg, L104EA29TIg, or L104EA29WIg. The sol. CTLA-4 mutant may administered in combination with immunosuppressive agent such as Rapamycin, anti-human IL2R monoclonal antibody, corticosteroid, cyclosporin, tarcolimus, prednisone, azathioprine, TOR inhibitor, methotrexate, TNF.alpha. blocker, TNF antagonist, infliximab, inflammatory cytokine-targeting biol. agent, hydroxychloroquine, sulphasalazopryine, gold salts, etanercept, or anakinra.

IT INDEXING IN PROGRESS

L14 ANSWER 2 OF 15 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:813967 HCAPLUS
DOCUMENT NUMBER: 137:304770
TITLE: Systems and methods for inducing mixed **chimerism**
INVENTOR(S): Hering, Bernard; Guo, Zhiguang
PATENT ASSIGNEE(S): Islet Technology, Inc., USA
SOURCE: PCT Int. Appl., 103 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002083187	A1	20021024	WO 2002-US12255	20020416

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR,

BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
PRIORITY APPLN. INFO.: US 2001-284005P P 20010416
US 2001-855027 A 20010514

AB A mixed **chimeric** immune system is created for a variety of treatments and techniques. Mixed **chimerism** is established in a recipient without risk of profound neutropenia or **graft** -vs.-host-disease (GVHD) by administering a cell **transplant** from a donor to a recipient along with a conditioning treatment and an immune blockade treatment. The invention relates to inducing tolerance to **transplanted** materials such as allogeneic, xenogeneic, and autogeneic materials **transplanted** into a patient and to restoring self-tolerance in the case of autoimmunity conditions. More specifically, the invention relates to creating mixed **chimerism** in patients and treating **graft** rejection, malignant cell growth, and autoimmune conditions.

IT **55-98-1**, Busulfan
RL: PAC (Pharmacological activity); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(systems and methods for inducing mixed **chimerism** in the immune system for inducing tolerance to **transplanted** materials)

REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 3 OF 15 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:923628 HCAPLUS
DOCUMENT NUMBER: 136:52724
TITLE: Methods for regulating a cell-mediated immune response by blocking lymphocytic signals and by blocking LFA-1 mediated adhesion
INVENTOR(S): Townsend, Robert M.; Todderud, Charles Gordon; Peach, Robert J.
PATENT ASSIGNEE(S): Bristol-Myers Squibb Company, USA
SOURCE: PCT Int. Appl., 75 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001095928	A2	20011220	WO 2001-US18619	20010608
WO 2001095928	A3	20020530		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

US 2002039577	A1	20020404	US 2001-877987	20010608

PRIORITY APPLN. INFO.: US 2000-210671P P 20000609

AB The invention provides methods for regulating cell-mediated immune responses, immune system diseases and allograft **transplant** rejection by interfering with the interaction of at least three different cell surface mols. with their natural ligands. A first cellular

interaction is mediated by CD28/B7/**CTLA4**, a second interaction is mediated by CD40/CD154, and a third interaction is mediated by LFA-1 interaction with its ligands. Regulation of a cell-mediated immune response affects immune system diseases such as those assocd. with allograft **transplantation**.

L14 ANSWER 4 OF 15 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:850964 HCAPLUS
DOCUMENT NUMBER: 135:370653
TITLE: Compositions and methods for achieving immune suppression
INVENTOR(S): Strom, Terry B.; Maslinski, Wlodzimierz; Zheng, Xin Xiao; Kim, Yon Su; Lacraz, Sylvie Ferrari
PATENT ASSIGNEE(S): Beth Israel Deaconess Medical Center, Inc., USA
SOURCE: PCT Int. Appl., 42 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001087330	A2	20011122	WO 2001-US15578	20010514
WO 2001087330	A3	20020530		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

US 2002128436	A1	20020912	US 2001-855313	20010514

PRIORITY APPLN. INFO.: US 2000-203801P P 20000512

AB Compn. comprising a first agent that targets an interleukin-15 receptor (IL-15R) and a second agent that inhibits a costimulatory signal transmitted between a T cell and an antigen-presenting cell (APC). The first agent is mutant IL-15, **chimeric** IL-15 or anti-IL15R antibody; and the second agent is B7 antigen, B7-1 or B7-2 antigen, anti-B7 antibody or **chimeric CTLA4**/Ig. Said compn. may be used for the diagnosis and therapy of immune diseases, esp. autoimmune diseases.

L14 ANSWER 5 OF 15 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:816872 HCAPLUS
DOCUMENT NUMBER: 135:355016
TITLE: The use of tolerogenic dendritic cells for enhancing tolerogenicity in a host and methods for making the same
INVENTOR(S): Robbins, Paul D.; Lu, Lina; Giannoukakis, Nick
PATENT ASSIGNEE(S): University of Pittsburgh of the Commonwealth System of Higher Education, USA
SOURCE: PCT Int. Appl., 64 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001083713	A2	20011108	WO 2001-US13661	20010427
WO 2001083713	A3	20020314		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

US 2002048564	A1	20020425	US 2001-844915	20010427

PRIORITY APPLN. INFO.: US 2000-200479P P 20000428

AB The present invention relates to a tolerogenic mammalian dendritic cells (DCs) and methods for the prodn. of the tolerogenic DCs. In addn., the present invention provides a method for enhancing tolerogenicity in a host comprising administering the tolerogenic mammalian DCs of the present invention to the host. The tolerogenic DCs of the present invention comprise an oligodeoxyribonucleotide (ODN) which has one or more NF-.kappa.B binding sites. The tolerogenic DCs of the present invention may further comprise a viral vector, and preferably an adenoviral vector, which does not affect the tolerogenicity of the tolerogenic DCs when present therein. Enhanced tolerogenicity in a host is useful for prolonging foreign **graft** survival and for treating inflammatory related diseases, such as autoimmune diseases.

L14 ANSWER 6 OF 15 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER:	2000:763245 HCAPLUS
DOCUMENT NUMBER:	134:50996
TITLE:	Comparison of different busulfan analogues for depletion of hematopoietic stem cells and promotion of donor-type **chimerism** in murine bone marrow **transplant** recipients
AUTHOR(S):	Westerhof, G. Robbin; Ploemacher, Rob E.; Boudewijn, Adrie; Blokland, Irene; Dillingh, Jan H.; McGown, Alan T.; Hadfield, John A.; Dawson, Martin J.; Down, Julian D.
CORPORATE SOURCE:	Department of Hematology, Erasmus University, Rotterdam, 3000 DR, Neth.
SOURCE:	Cancer Research (2000), 60(19), 5470-5478 CODEN: CNREA8; ISSN: 0008-5472
PUBLISHER:	American Association for Cancer Research
DOCUMENT TYPE:	Journal
LANGUAGE:	English

AB Busulfan (1,4-butanediol dimethanesulfonate, BU) is relatively unique among other std. chemotherapy compds. in its ability to deplete noncycling primitive stem cells in the host and consequently to allow for high levels of long-term, donor-type engraftment after bone marrow **transplantation** (BMT). Such a property explains why this drug can be used as an alternative to total body irradn. in preparative regimes for BMT. However, as with radiation, BU conditioning is still troubled by severe toxicities that limit its applications to suboptimal drug doses. These problems stress the need for other BMT-conditioning drugs that are better tolerated and more selectively targeted toward normal and malignant hematopoietic stem cells. We have therefore compared the effects of

various novel dimethanesulfonate compds. (related to BU) in terms of their toxicity to different stem cell subsets in vivo and in vitro and their ability to provide for long-term donor bone marrow engraftment using the congenic **glucose**-6-phosphate isomerase type 1 marker. Introduction of a benzene or cyclohexane ring in some of these drugs affords rigidity to the mol. and restricts the spatial positioning of the alkylating groups. Among 25 different compds. thus far tested at single doses, PL63 [cis-1,2-(2-hydroxyethyl) cyclohexane dimethanesulfonate] proved to be the most effective in providing for hematopoietic engraftment. The trans-isomer of the same compd. gave significantly less engraftment and was comparable with the effects of dimethylbusulfan and Hepsulfam. The engraftment data correlated well with the depletion of different bone marrow stem cell subsets in the host as measured using the cobblestone area forming cell assay. The extent of stem cell depletion could not be explained on the basis of the distance and orientation of the two alkylating groups. Pharmacokinetic data, however, indicate that there is a correlation between biol. activity and plasma levels reached. The diverse cytotoxic effects shown by these novel analogs of BU have provided a basis for relating biol. activity with pharmacokinetic properties rather than with structural properties such as distance and orientation of the two alkylating groups. The identification of highly active compds. such as PL63 offers an opportunity for further developing other closely related drugs for potential application in clin. BMT conditioning therapy.

IT **55-98-1**, Busulfan
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(comparison of different busulfan analogs for depletion of hematopoietic stem cells and promotion of donor-type **chimerism** in murine bone marrow **transplant** recipients)

REFERENCE COUNT: 50 THERE ARE 50 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 7 OF 15 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:723165 HCAPLUS
DOCUMENT NUMBER: 131:350247
TITLE: Immunosuppression by blocking t cell co-stimulation signal 2 (b7/cd28 interaction)
INVENTOR(S): Lechler, Ian Robert; Dorling, Anthony
PATENT ASSIGNEE(S): Imperial College Innovations Limited, UK
SOURCE: PCT Int. Appl., 43 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9957266	A2	19991111	WO 1999-GB1350	19990430
WO 9957266	A3	20000420		

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG,

CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

CA 2326671	AA	19991111	CA 1999-2326671	19990430
AU 9937213	A1	19991123	AU 1999-37213	19990430
EP 1073737	A2	20010207	EP 1999-919418	19990430

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI

PRIORITY APPLN. INFO.: GB 1998-9280 A 19980430
WO 1999-GB1350 W 19990430

AB The invention provides means and methods for inhibiting T-cell mediated rejection of a xenotransplanted organ by blocking the delivery of co-stimulatory signal 2 (the B7/CD28 interaction) in order to prevent the activation of xenoreactive T-cells in the recipient. In a first aspect, co-stimulation is prevented by administration to the organ recipient of a sol. form of CTLA-4 from the xenogeneic donor organism. This preferentially binds B7 on the xenograft and blocks the interaction between B7 on the xenogeneic donor cells and CD28 on recipient T-cells. In a second aspect, co-stimulation is antagonized by expressing a ligand for CTLA-4 on the xenogeneic donor cells. This ligand binds to CTLA-4 on activated T-cells of the recipient and antagonizes signal 2. In a third aspect, co-stimulation is prevented by expressing recipient organism MHC class II on the surface of the cells of the xenogeneic donor organ. The donor cells are thus able to present xenoantigens to a recipient T-cell in the context of self-MHC class II. If the donor cells do not express B7, or if B7 is blocked, the xenoreactive recipient T-cell becomes anergic.

IT **250243-85-7**, CTLA-4 (antigen) (human)
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(amino acid sequence; immunosuppression by blocking t cell co-stimulation signal 2 (b7/cd28 interaction))

L14 ANSWER 8 OF 15 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:640667 HCAPLUS
DOCUMENT NUMBER: 131:262585
TITLE: Method of inhibiting immune system destruction of **transplanted** viable cells by cell encapsulation and immunosuppressants
INVENTOR(S): Weber, Collin J.; Hagler, Mary K.; Linsley, Peter S.; Kapp, Judith A.; Safely, Susan A.
PATENT ASSIGNEE(S): Emory University, USA; Bristol Myers-Squibb Co.
SOURCE: PCT Int. Appl., 159 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9949734	A1	19991007	WO 1999-US6630	19990326

W: AU, CA, JP
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

AU 9932067	A1	19991018	AU 1999-32067	19990326
EP 984699	A1	20000315	EP 1999-914166	19990326

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI

JP 2002501548	T2	20020115	JP 1999-549567	19990326

PRIORITY APPLN. INFO.: US 1998-49865 A 19980327
WO 1999-US6630 W 19990326

Searched by M. Smith

AB This invention provides a method of preventing or inhibiting destruction of viable cell **transplants** by the subject's immune system by: (a) encapsulation of the viable cells, or tissue comprising the viable cells, prior to **transplantation** within a semipermeable membrane; and (b) treating the subject with a substance which inhibits an immune-system costimulation event. In one embodiment, the substance which inhibits an immune-system costimulation event is **CTLA4**. Thus, a method of treating insulin-dependent **diabetes** in a subject comprises: (a) microencapsulation of viable insulin-producing cells within a poly-L-lysine-alginate semipermeable membrane; (b) **transplanting** an effective amt. of such microencapsulated cells into the subject; and (c) treating the subject with an effective amt. of an immunosuppressant, such as **CTLA4**-Ig. Neither microencapsulation nor **CTLA4** -Ig alone prevented destruction of neonatal porcine **islets** in spontaneously **diabetic** NOD mice. However, a synergy between **CTLA4**-Ig treatment of NOD mice and **islet** encapsulation was obsd. which prolonged discordant **islet** xenograft survival to 10 days compared to 8 days for encapsulated cells and 3 days for the **CTLA4**-Ig treatment alone.

REFERENCE COUNT: 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 9 OF 15 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:622174 HCAPLUS
DOCUMENT NUMBER: 131:238812
TITLE: Methods and compounds for prevention of **graft** rejection
INVENTOR(S): Strom, Terry; Libermann, Towia
PATENT ASSIGNEE(S): Beth Israel Hospital Association, USA
SOURCE: U.S., 37 pp., Cont.-in-part of U.S. Ser. No. 24,569, abandoned.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5958403	A	19990928	US 1994-273402	19940711
EP 1175910	A2	20020130	EP 2001-114693	19930301
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE				
US 2002164311	A1	20021107	US 2001-804717	20010312

PRIORITY APPLN. INFO.:
US 1992-843731 B2 19920228
US 1993-24569 B2 19930301
EP 1993-907073 A3 19930301
US 1994-273402 A1 19940711

AB Disclosed is a method of localized immunosuppression which may be used for preventing **graft** rejection or for preventing tissue destruction due to autoimmune disease. Also disclosed is a protein suppressor factor (IL-2.15) that is secreted by cloned anergic T-cells, blocks interleukin 2 (IL-2) stimulated T-cell proliferation, has an apparent mol. wt. of between 10 and 30 kDa, can be inactivated by heating to 65.degree. C. for 15 min, blocks interleukin 4 (IL-4) stimulated T-cell proliferation in vitro, is non-cytotoxic to T-cells, and does not inhibit the prodn. of IL-2 by T-cells in vitro. **Graft** rejection is prevented by inducing a state of local immunosuppression at the **transplant** site with expression of recombinant proteins by the allograft. According to the claims, the method comprises: (a) introducing in an **islet**

cell, ex vivo, a nucleic acid sequence encoding **CTLA4**-Ig operably linked to a promoter, wherein the **CTLA4**-Ig is expressed by the **islet** cell; and (b) **transplanting** the **islet** cell into the patient, wherein **CTLA4**-Ig is expressed at a level sufficient to inhibit the rejection of the **transplanted** cell.

REFERENCE COUNT: 53 THERE ARE 53 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 10 OF 15 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:789047 HCAPLUS
DOCUMENT NUMBER: 130:24115
TITLE: Use of a CD40:CD154 binding interrupter to prevent counter adaptive immune responses, particularly **graft** rejection
INVENTOR(S): Kirk, Allan D.; Harlan, David M.; Thomas, David; Kauffman, Michael; Burkly, Linda
PATENT ASSIGNEE(S): Biogen, Inc., USA
SOURCE: PCT Int. Appl., 37 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9852606	A1	19981126	WO 1998-US10075	19980515
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, US, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9874940	A1	19981211	AU 1998-74940	19980515
AU 735592	B2	20010712		
EP 980259	A1	20000223	EP 1998-922381	19980515
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
BR 9809641	A	20000711	BR 1998-9641	19980515
JP 2002500648	T2	20020108	JP 1998-550477	19980515
WO 9856417	A1	19981217	WO 1998-US11910	19980610
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9879567	A1	19981230	AU 1998-79567	19980610
AU 748533	B2	20020606		
EP 1009432	A1	20000621	EP 1998-930097	19980610
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2002504120	T2	20020205	JP 1999-503086	19980610

NO 9905617	A	20000117	NO 1999-5617	19991116
US 2002119150	A1	20020829	US 2002-120272	20020409

PRIORITY APPLN. INFO.:

US 1997-46791P	P	19970517
US 1997-49389P	P	19970611
US 1998-85145P	P	19980512
WO 1998-US10075	W	19980515
WO 1998-US11910	W	19980610
US 1999-442012	B1	19991117

AB Compns. and methods disclosed herein capitalize on the discovery that rejection of a tissue **graft** can be inhibited using a CD40:CD154 binding interrupter, either alone or in combination with another immunomodulator or immunosuppressor. An advantageous, synergistic combination includes a CD40:CD154 binding interrupter and a CD28 signalling interrupter. An exemplary CD40:CD154 binding interrupter is an anti-CD154 monoclonal antibody, such as an antibody having the antigen-specific binding characteristics of the 5c8 monoclonal antibody. An exemplary CD28 signalling interrupter is a **CTLA4**-Ig fusion protein. The disclosed compns. and methods unexpectedly can be used to prolong survival of **grafted** tissue in a recipient host, to reverse acute **graft** rejection, and to attenuate immunol. consequences of the failure of **grafted** tissue.

REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 11 OF 15 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:684303 HCAPLUS
DOCUMENT NUMBER: 127:358050
TITLE: Novel product and process for T lymphocyte veto
INVENTOR(S): Staerz, Uwe D.
PATENT ASSIGNEE(S): National Jewish Center for Immunology and Respiratory Medicine, USA; Staerz, Uwe D.
SOURCE: PCT Int. Appl., 116 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9737687	A1	19971016	WO 1997-US5943	19970410
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
US 6060054	A	20000509	US 1996-630172	19960410
CA 2251819	AA	19971016	CA 1997-2251819	19970410
AU 9727258	A1	19971029	AU 1997-27258	19970410
EP 929316	A1	19990721	EP 1997-921134	19970410
R: CH, DE, FR, GB, IT, LI, SE				
US 6264950	B1	20010724	US 1999-375419	19990817

PRIORITY APPLN. INFO.:

US 1996-630172	A2	19960410
WO 1997-US5943	W	19970410

AB The present invention relates to a product and process for suppressing an immune response using a T lymphocyte veto mol. capable of blocking cell

surface mols. responsible for T cell activation. Disclosed is a CD4 or CD2 mol., assocd. with an Ig mol. capable of binding to a major histocompatibility antigen. The CD2 or CD4 mol. may also be replaced by **CTLA4**, Fas ligand, CD5, CD7, CD9, CD11, CD18, CD27, CD43, CD45. CD48, B7.1 or B7.2 protein. Also disclosed is a method to produce a T lymphocyte veto mol., a therapeutic compn. comprising a T lymphocyte veto mol. and methods to use T lymphocyte veto mols. in therapeutic processes requiring suppression of an immune response.

L14 ANSWER 12 OF 15 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:575682 HCAPLUS
DOCUMENT NUMBER: 127:261655
TITLE: Distinct mechanisms for the induction and maintenance of allograft tolerance with **CTLA4**-Fc treatment
AUTHOR(S): Tran, Hanh M.; Nickerson, Peter W.; Restifo, Anthony C.; Ivis-Woodward, Maria A.; Patel, Anita; Allen, Richard D.; Strom, Terry B.; O'Connell, Philip J.
CORPORATE SOURCE: National Pancreas Transplant Unit, Westmead Hospital, Westmead, Australia
SOURCE: Journal of Immunology (1997), 159(5), 2232-2239
CODEN: JOIMA3; ISSN: 0022-1767
PUBLISHER: American Association of Immunologists
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A murine **CTLA4**/Fc.gamma.2a heavy chain (**mCTLA4**-Fc) **chimeric** fusion mol. was used in B6AF1 recipients of BALB/c **pancreatic islet** allografts to study the induction and maintenance of tolerance following inhibition of the CD28-B7 pathway for T cell activation. Donor-specific tolerance was achieved by administering 100 .mu.g of **mCTLA4**-Fc on alternate days for 14 days (8 total doses) or a single 500 .mu.g dose of **mCTLA4**-Fc on day 2 after **transplant**. Tolerance was mediated by long-lived peripheral lymphocytes and showed features of organ and alloantigen specificity. Whereas tolerance could not be established in allograft recipients receiving simultaneous **mCTLA4**-Fc and rIL-2, previously tolerant animals did not reject their **grafts** when given IL-2, suggesting that the induction and maintenance phases of tolerance were distinct and sep. The maintenance of donor-specific tolerance was an active immunol. process that was CD4+ T cell dependent and could be adoptively transferred to naive lymphocytes, but could not be explained by apoptosis or deletion of alloreactive T cells. Although an IL-2-sensitive mechanism such as anergy may contribute toward the induction of tolerance, its maintenance involves active suppression.

L14 ANSWER 13 OF 15 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:536915 HCAPLUS
DOCUMENT NUMBER: 127:219551
TITLE: Antibodies and immunoglobulin fusion proteins having modified effector functions and uses therefor
INVENTOR(S): Gray, Gary S.; Carson, Jerry; Javaherian, Kashi; Jellis, Cindy L.; Rennert, Paul D.; Silver, Sandra
PATENT ASSIGNEE(S): Repligen Corp., USA; Gray, Gary S.; Carson, Jerry; Javaherian, Kashi; Jellis, Cindy L.; Rennert, Paul D.; Silver, Sandra
SOURCE: PCT Int. Appl., 104 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9728267	A1	19970807	WO 1997-US1698	19970203
W: AU, CA, JP, US				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
CA 2243986	AA	19970807	CA 1997-2243986	19970203
AU 9722554	A1	19970822	AU 1997-22554	19970203
EP 877812	A1	19981118	EP 1997-905730	19970203
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2000504564	T2	20000418	JP 1997-527885	19970203
US 6444792	B1	20020903	US 1999-227595	19990108
US 2002114814	A1	20020822	US 2001-27075	20011220

PRIORITY APPLN. INFO.: US 1996-595590 A2 19960202
WO 1997-US1698 W 19970203
US 1999-227595 A1 19990108

AB **CTLA4**-Ig fusion proteins having modified Ig const. region-mediated effector functions, and nucleic acids encoding the fusion proteins, are described. The **CTLA4**-Ig fusion proteins comprise two components: a first peptide having a **CTLA4** activity and a second peptide comprising an Ig const. region which is modified to reduce at least one const. region-mediated biol. effector function relative to a **CTLA4**-IgG1 fusion protein. The nucleic acids of the invention can be integrated into various expression vectors, which in turn can direct the synthesis of the corresponding proteins in a variety of hosts, particularly eukaryotic cells. The **CTLA4**-Ig fusion proteins described herein can be administered to a subject to inhibit an interaction between a **CTLA4** ligand (e.g., B7-1 and/or B7-2) on an antigen presenting cell and a receptor for the **CTLA4** ligand (e.g., CD28 and/or **CTLA4**) on the surface of T cells to thereby suppress an immune response in the subject, for example to inhibit **transplantation** rejection, **graft** vs. host disease or autoimmune responses.

L14 ANSWER 14 OF 15 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1995:711486 HCAPLUS
DOCUMENT NUMBER: 123:110099
TITLE: Ex vivo coating of **islet** cell allografts with murine **CTLA4**/Fc promotes **graft** tolerance
AUTHOR(S): Steurer, Wolfgang; Nickerson, Peter W.; Steele, Alan W.; Steiger, Jurg; Zheng, Xin Xiao; Strom, Terry B.
CORPORATE SOURCE: Harvard Med. Sch., Beth Israel Hosp., Boston, MA, 02215, USA
SOURCE: Journal of Immunology (1995), 155(3), 1165-74
CODEN: JOIMA3; ISSN: 0022-1767
PUBLISHER: American Association of Immunologists
DOCUMENT TYPE: Journal
LANGUAGE: English

AB To test the hypothesis that blockade of B7-triggered costimulation by donor cells could preclude allograft rejection, the authors coated crude **islet** allograft prepns. in vitro for 1 h with a murine **CTLA4**/Fc fusion protein. Murine **CTLA4**/Fc blocks the proliferative response in primary mixed lymphocyte cultures (MLC) and Con A-stimulated murine spleen cell cultures by 85-95%. Responder cells from a primary MLC contg. **mCTLA4**/Fc were hyporesponsive upon

restimulation to the same stimulator cells in a secondary MLC lacking **mCTLA4**/Fc. Because of mutations in the Fc.gamma.RI and C'1q binding sites of the Fc portion of the murine **CTLA4**/Fc fusion protein, the mol. binds to, but does not target, cells for Ab-dependent cellular cytotoxicity or complement-directed cytolysis. Although systemic immunosuppression was not applied, 42% (10 of 24) of B6AF1 recipients of **islet** allografts pretreated with **CTLA4**/Fc were permanently engrafted. Further, 50% of hosts bearing functioning **islet** allografts >150 days post-**transplant** were formally proved to be tolerant to donor tissues. A persistent CD4+ and CD8+ T cell infiltrate surrounding, but not invading, **islet grafts** in tolerant hosts was discerned. In control expts., 89% (8 of 9) of **islet** allografts coated with mIgG3, and 100% pretreated with media alone were rejected. Thus, (1) B7-triggered costimulation by donor APCs is an important element of rejection, and (2) blockade of the B7 pathway by in vitro allograft manipulation is able to induce tolerance.

L14 ANSWER 15 OF 15 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1994:672171 HCAPLUS
DOCUMENT NUMBER: 121:272171
TITLE: Methods for regulating the immune response using **CTLA4**-binding molecules and IL4-binding molecules
INVENTOR(S): Linsley, Peter S.; Ledbetter, Jeffrey A.; Damle, Nitin K.; Brady, William; Wallace, Philip M.
PATENT ASSIGNEE(S): Bristol-Myers Squibb Co., USA
SOURCE: Can. Pat. Appl., 94 pp.
CODEN: CPXXEB
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 8
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
CA 2113744	AA	19940723	CA 1994-2113744	19940119
US 5770197	A	19980623	US 1993-8898	19930122

PRIORITY APPLN. INFO.: US 1993-8898 A 19930122
US 1991-723617 B2 19910627

AB A compn. comprising a **CTLA4**-binding mol. and an IL4-binding mol. for use in regulating an immune response by blocking a B7 interaction with lymphocytes is claimed. The use of the compn. for regulating the immune response, e.g., for inhibiting antibody prodn. or cell-mediated immunity, for inhibiting tissue **transplant** rejection, and for inhibiting **graft**-vs.-host disease is further claimed. The **CTLA4** receptor was identified as a ligand for the B7 antigen. The complete amino acid sequence encoding human **CTLA4** receptor gene was detd.. The sequence differs at position 111 from the previously reported (Dariavach, 1988) sequence. In the present sequence, residue 111 is threonine and it is part of a newly identified N-linked glycosylation site. A CHO cell line producing **CTLA4** as an Ig fusion protein was prepd. A dissocn. const. of 12 nM for binding of B7 antigen to **CTLA4**-Ig fusion protein was obsd. The **CTLA4**-Ig **chimera** was a potent inhibitor in vitro of T and B lymphocyte response. In vivo, the protein significantly prolonged human **islet graft** survival in mice in a donor-specific manner.

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show files
File 155:MEDLINE(R) 1966-2002/Nov W4
File   5:Biosis Previews(R) 1969-2002/Dec W4
         (c) 2002 BIOSIS
File  34:SciSearch(R) Cited Ref Sci 1990-2002/Dec W5
         (c) 2002 Inst for Sci Info
File  35:Dissertation Abs Online 1861-2002/Nov
         (c) 2002 ProQuest Info&Learning
File  65:Inside Conferences 1993-2002/Dec W4
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File  71:ELSEVIER BIOBASE 1994-2002/Dec W4
         (c) 2002  Elsevier Science B.V.
File  73:EMBASE 1974-2002/Dec W4
         (c) 2002 Elsevier Science B.V.
File  94:JICST-EPlus 1985-2002/Oct W3
         (c)2002 Japan Science and Tech Corp(JST)
File 128:PHARMAPROJECTS 1980-2002/Dec W3
         (c) 2002 PJB Publications,Ltd.
File 144:Pascal 1973-2002/Dec W4
         (c) 2002 INIST/CNRS
File 149:TGG Health&Wellness DB(SM) 1976-2002/Dec W2
         (c) 2002 The Gale Group
File 340:CLAIMS(R)/US Patent 1950-02/Dec 19
         (c) 2002 IFI/CLAIMS(R)
File 351:Derwent WPI 1963-2002/UD,UM &UP=200282
         (c) 2002  Thomson Derwent
File 357:Derwent Biotech Res. _1982-2002/Dec W4
         (c) 2002 Thomson Derwent & ISI
File 440:Current Contents Search(R) 1990-2002/Dec 27
         (c) 2002 Inst for Sci Info
?ds

Set     Items   Description
S1        771   (BUSULFAN? OR CTLA4?) AND (PANCRE? OR ISLET? OR DIABET? OR
                 GLUCOSE(W)TOLER? OR INTOLER?) AND (GRAFT? OR TRANSPLAN?)
S2        407   RD (unique items)
S3        315   (BUSULFAN? OR CTLA4?) (S) (PANCRE? OR ISLET? OR DIABET? OR
                 GLUCOSE(W)TOLER? OR INTOLER?) (S) (GRAFT? OR TRANSPLAN?)
S4        756   (BUSULFAN? OR CTLA4?) AND (PANCRE? OR ISLET? OR DIABET? OR
                 GLUCOSE(W)INTOLER?) AND (GRAFT? OR TRANSPLAN?)
S5        313   (BUSULFAN? OR CTLA4?) (S)(PANCRE? OR ISLET? OR DIABET? OR -
                 GLUCOSE(W)INTOLER?) (S) (GRAFT? OR TRANSPLAN?)
S6        104   RD (unique items)
S7         15   S6 AND CHIMER?
?t7/7/1-15

 7/7/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12959430   21885000   PMID: 11888156
  Islet cell transplantation tolerance.
  Rossini A A; Mordes J P; Greiner D L; Stoff J S
  Department of Medicine, University of Massachusetts Medical School,
Worcester 01655, USA. aldo.rossini@umassmed.edu
  Transplantation (United States)  Oct 27 2001,  72  (8 Suppl)  pS43-6,
ISSN 0041-1337   Journal Code: 0132144
  Contract/Grant No.:  AI42669; AI; NIAID; DK/AI53006; DK; NIDDK; DK32520;
DK; NIDDK
  Document type: Journal Article; Review; Review, Tutorial
  Languages: ENGLISH
  Main Citation Owner: NLM
  Record type: Completed
```

Curative islet transplantation for type 1 diabetes currently requires lifelong systemic immunosuppression. Induction of islet transplantation tolerance would be far preferable. We have previously demonstrated that blockade of costimulation by the administration of a donor-specific transfusion in combination with anti-CD154 monoclonal antibody leads to permanent islet and prolonged skin allograft survival in mice. The protocol requires the presence of CD4+ T cells, interferon-gamma, and CTLA4 , and involves the deletion of CD8+ alloreactive T cells. Translation of this strategy into clinical practice will, however, require attention to at least two issues. First, we have observed that the presence of viral infection during tolerance interferes with tolerance induction. Second, we have observed that our tolerance induction protocol is ineffective in autoimmune nonobese diabetic mice. We hypothesize that resistance to tolerance induction in nonobese diabetic mice is due to the presence of memory autoreactive cells. To overcome the deleterious effects of viral infection and of primed memory responses, it may be necessary to modify current tolerance induction strategies based on costimulatory blockade. These modifications may require patient isolation, the generation of hematopoietic chimerism , or treatments that target the specific T-cell populations, cytokines, and/or costimulatory factors responsible for resistance. Such modifications may make it possible to extend tolerance induction to the "real world" situation of individuals with type 1 diabetes who are likely to harbor both memory allo-and autoreactive immune cells. (24 Refs.)

Record Date Created: 20020312

7/7/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09508293 97422543 PMID: 9278311

Distinct mechanisms for the induction and maintenance of allograft tolerance with CTLA4-Fc treatment.

Tran H M; Nickerson P W; Restifo A C; Ivis-Woodward M A; Patel A; Allen R D; Strom T B; O'Connell P J

National Pancreas Transplant Unit, Westmead Hospital, New South Wales, Australia.

Journal of immunology (Baltimore, Md. : 1950) (UNITED STATES) Sep 1 1997, 159 (5) p2232-9, ISSN 0022-1767 Journal Code: 2985117R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

A murine CTLA4 /Fc gamma2a heavy chain (mCTLA4-Fc) chimeric fusion molecule was used in B6AF1 recipients of BALB/c pancreatic islet allografts to study the induction and maintenance of tolerance following inhibition of the CD28-B7 pathway for T cell activation. Donor-specific tolerance was achieved by administering 100 microg of mCTLA4-Fc on alternate days for 14 days (8 total doses) or a single 500 microg dose of mCTLA4-Fc on day 2 after transplant . Tolerance was mediated by long-lived peripheral lymphocytes and showed features of organ and alloantigen specificity. Whereas tolerance could not be established in allograft recipients receiving simultaneous mCTLA4-Fc and rIL-2, previously tolerant animals did not reject their grafts when given IL-2, suggesting that the induction and maintenance phases of tolerance were distinct and separate. The maintenance of donor-specific tolerance was an active immunologic process that was CD4+ T cell dependent and could be adoptively transferred to naive lymphocytes, but could not be explained by apoptosis or deletion of alloreactive T cells. Although an IL-2-sensitive mechanism such as anergy may contribute toward the induction of tolerance, its maintenance involves active suppression.

Record Date Created: 19970919

7/7/3 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11297014 BIOSIS NO.: 199800078346
Short-term suppression of the xeno-immune response with mCTLA4-Fc treatment.
AUTHOR: Tran Hanh M; Nickerson Peter W; Patel Anita; Strom Terry B; Allen Richard D M; O'Connell Philip J(a)
AUTHOR ADDRESS: (a)Natl. Pancreas Transplantation Unit, Westmead Hosp., Westmead, NSW 2145**Australia
JOURNAL: Xenotransplantation 4 (4):p222-227 Nov., 1997
ISSN: 0908-665X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A murine CTLA4 -Fc chimeric fusion protein was used to determine if inhibition of the CD28-B7 pathway for T-cell activation could result in prolonged or indefinite survival of pancreatic islet xenografts in mice. B6AF1 recipients of Wistar rat pancreatic islet xenografts that received short-term mCTLA4-Fc treatment had prolonged graft survival (28 days vs. 9 days) but all animals rejected their grafts . This survival advantage was similar to that achieved with short-term depletion of CD3+ or CD4+ T cells with 145.2C11 (median graft survival 21 days) or GK 1.5 mAb (median graft survival 33 days), respectively. Combined GK1.5, 145.2C11, and mCTLA4-Fc treatment for the first 2 weeks post- transplant and maintenance therapy with GK1.5 and mCTLA4-Fc for the next 4 weeks produced the best results (median survival 63 days). However, islet xenografts were rapidly rejected upon cessation of treatment. Unlike in allografts, short-term inhibition of the CD28-B7 pathway with mCTLA4Fc did not result in long-term rat xenograft survival. This suggests that the conditions necessary for quenching xenograft rejection and inducing tolerance differ significantly from those found in allotransplantation and acquired xenograft tolerance may be difficult to achieve.

7/7/4 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

09069535 Genuine Article#: 363BW Number of References: 30
Title: Costimulatory blockade by the induction of an endogenous xenospecific antibody response
Author(s): Rogers NJ; Mirenda V; Jackson I; Dorling A; Lechler RI (REPRINT)
Corporate Source: UNIV LONDON IMPERIAL COLL SCI TECHNOL & MED,DEPT IMMUNOL, HAMMERSMITH CAMPUS, DU CANE RD/LONDON W12 0NN//ENGLAND/ (REPRINT); UNIV LONDON IMPERIAL COLL SCI TECHNOL & MED,DEPT IMMUNOL/LONDON W12 0NN//ENGLAND/
Journal: NATURE IMMUNOLOGY, 2000, V1, N2 (AUG), P163-168
ISSN: 1529-2908 Publication date: 20000800
Publisher: NATURE AMERICA INC, 345 PARK AVE SOUTH, NEW YORK, NY 10010-1707
Language: English Document Type: ARTICLE
Abstract: Xenogeneic tissues induce vigorous T cell immunity, reflecting the ability of costimulatory molecules to function across species barriers. We describe a strategy to inhibit costimulation that exploits species differences using the model of porcine pancreatic islet transplantation into mice. Mice were immunized with chimeric peptides

that contained a known T cell epitope and selected sequences of the porcine costimulatory molecule CD86. This resulted in anti-peptide antibody responses that recognized intact porcine CD86, blocked costimulation by porcine CD86 but not murine CD86 in vitro, and prolonged the survival of porcine islet grafts in vivo. This strategy of inducing endogenous donor-specific costimulatory blockade has potential clinical applicability.

7/7/5 (Item 2 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

09052228 Genuine Article#: 361EV Number of References: 24
Title: Local production of anti-CD4 antibody by transgenic allogeneic grafts affords partial protection
Author(s): Zhan YF; Martin RM; Sutherland RM; Brady JL; Lew AM (REPRINT)
Corporate Source: ROYAL MELBOURNE HOSP,WALTER & ELIZA HALL INST MED RES/PARKVILLE/VIC 3050/AUSTRALIA/ (REPRINT); ROYAL MELBOURNE HOSP,WALTER & ELIZA HALL INST MED RES/PARKVILLE/VIC 3050/AUSTRALIA/
Journal: TRANSPLANTATION, 2000, V70, N6 (SEP 27), P947-954
ISSN: 0041-1337 Publication date: 20000927
Publisher: LIPPINCOTT WILLIAMS & WILKINS, 530 WALNUT ST, PHILADELPHIA, PA 19106-3621
Language: English Document Type: ARTICLE
Abstract: Background. Immunosuppressive drugs and antilymphocyte antibody are used clinically to suppress cellular rejection responses. However, these systemic regimens often led to general immunodeficiency and thus increased susceptibility to opportunistic infection and neoplasia, Immunosuppressive molecules delivered locally may be a way of inhibiting rejection responses, whereas systemic immunity is preserved, To achieve protective local immunosuppression, we produced a graft secreting its own immunomodulator, by deriving transgenic mice expressing a chimeric anti-CD4 antibody (GK2c) in the pancreas.

Methods and Results. Transgenic mice in bm1 genetic background expressing a modified anti-mouse CD4 antibody (GK2c) under two promoters have been produced. Tissue expression of GK2c was detected by immunoperoxidase staining, Under the cytomegalovirus promoter, there was abundant GK2c expression in pancreatic exocrine tissue. Under the rat preproinsulin II promoter, there was abundant GK2e expression in pancreatic endocrine tissue only. High-expression transgenic lines had 10-100 mu g/ml GK2c in blood plasma. By flow cytometry, these transgenic mice were devoid of CD4(+) cells in their peripheral lymphoid organs. To test transgenic mice as donors, fetal pancreata from transgenic mice were grafted into fully allogeneic CBA mice under the kidney capsule, transgenic grafts had prolonged survival compared with control non-transgenic grafts. Furthermore, GK2c transgenic grafts had reduced infiltration with an absence of CD4(+) cells at the graft site without any effect on the cell composition in lymphatic tissues.

Conclusion. Transgenic grafts that secrete anti-CD4 antibody can afford some protection against graft rejection, while only affecting the CD4 population at the graft site.

7/7/6 (Item 1 from file: 149)
DIALOG(R)File 149:TGG Health&Wellness DB(SM)

01998559 SUPPLIER NUMBER: 75644220 (THIS IS THE FULL TEXT)
Advances in transplantation tolerance.
Yu, Xue-Zhong; Carpenter, Paul; Anasetti, Claudio

The Lancet, 357, 9272, 1959
June 16,
2001

TEXT:

Immunosuppressive drugs developed in the past two decades have improved the short-term survival of organ allografts, but tolerance has not been achieved and almost all transplant recipients continue to require drugs throughout life. Graft rejection arises from the cognate interaction of T cells with antigen-presenting cells, the recognition of alloantigen through the T-cell receptor, and the delivery of accessory stimulation signals. Once activated by the specific antigen, replicating T cells die if they are re-exposed to the same antigen. Since depletion of antigen-activated T cells is one critical mechanism of transplantation tolerance, drugs such as ciclosporin that interfere with activation-induced T-cell death could inhibit tolerance, whereas drugs such as mycophenolate mofetil, that induce the death of activated T cells, could facilitate tolerance. Other tolerance mechanisms depend on inactivation rather than elimination of allograft reactive T cells. When antigen recognition occurs without costimulation through the CD28 and CD154 accessory receptors, or in absence of cell division, T cells become unresponsive. Thus, inhibitors of CD28 and CD154, and inhibition of T-cell division by rapamycin promotes transplantation tolerance.

Haemopoietic stem cell transplantation and organ transplantation have become the standards of care for the treatment of various diseases. In the past 30 years, developments in immunogenetics and clinical immunosuppression have increased the use of and safety of transplant procedures. With rare exception, however, recipients of organ transplants require lifelong immunosuppression. By contrast, after a fairly short period of immunosuppressive therapy, recipients of haemopoietic stem cell transplants achieve mutual donor and recipient tolerance. The unique tolerogenic property of haemopoietic stem cell grafts depends on the ability of marrow-derived cells to colonise the recipient's thymus and induce T-cell tolerance to donor antigens by both deletional and non-deletional mechanisms.(1,2) It has been thought that tolerance to organ grafting in adults could not be achieved without transplantation of haemopoietic stem cells. However, greater understanding of the basic mechanisms of the immune response has facilitated development of therapeutic strategies to induce tolerance to vascularised organ grafts in mice without stem cell grafting or sustained immunosuppression.

Haemopoietic stem cell transplantation

In vivo T-cell depletion is sufficient to establish sustained allografts of MHC incompatible haemopoietic stem cells in rodents.(3) Chimeric animals are tolerant to organ grafts from the stem cell donor, but reject grafts from third party donors. Unfortunately, there are no reagents yet that can achieve in vivo T-cell depletion in human beings to a degree sufficient for engraftment of HLA incompatible stem cells, except for cytotoxic agents or total body irradiation.(4) The acquisition of large numbers of donor stem cells after mobilisation with granulocyte-colony stimulating factor and blood cell apheresis has contributed to improvement of engraftment. However, the toxicity of intense conditioning regimens contributes to transplant-related mortality and restricts use of the procedure to young patients.

It has been shown that mixed host: donor cell chimerism can be achieved despite use of low intensity regimens that are only moderately marrow toxic but are sufficiently immunosuppressive to establish donor grafts. This approach was first established in rodents--T-cell depleted recipients were transplanted with a mixture of host and donor T-cell depleted haemopoietic stem cells, and became tolerant mixed chimeras .(5) On the basis of this experiment and others, a clinical protocol was designed for transplantation of marrow cells from partly HLA incompatible donors in patients with lymphoma.(6) Recipients were conditioned with

cyclophosphamide and thymic irradiation, transplanted with T cell-replete donor marrow cells, and treated with antilymphocyte globulin, to deplete host and donor T cells in vivo, and then immunosuppressed with ciclosporin to inhibit activation of residual T cells. Engraftment was successful in four of the five cases.

A separate approach was developed in dogs. T cell- replete marrow transplants from MHC compatible littermates were able to establish durable mixed host:donor cell chimerism and tolerance in recipients conditioned with low dose total body irradiation (200 cGy) followed by postgrafting immunosuppression with ciclosporin and mycophenolate mofetil, a lymphocyte selective antimetabolite.(7) In this protocol ciclosporin and mycophenolate mofetil are critical to prevent graft rejection and graft-versus-host disease. To date, such a regimen has allowed successful engraftment of blood stem cells from HLA compatible siblings in 80% of 45 human recipients up to the age of 72 years who were treated for a variety of haematological disorders.(8) These and other initial clinical trials which used non-myeloablative regimens have succeeded at inducing recipient tolerance to donor alloantigens, allowing adoptive transfer of donor T cells with the purpose of eradicating leukaemia, lymphoma, and solid tumors.(9)

Prevention of organ graft rejection

The use of T cell-replete stem cell allografts requires partial or complete donor HLA matching, because otherwise acute or chronic graft-versus-host disease can occur. This procedure is therefore unsuitable for inducing donor-specific tolerance in clinical organ transplantation on a routine basis. Infusion of donor marrow cells without preconditioning has slightly decreased the rate of acute and chronic rejection of vascular allografts in human beings, but has not eliminated the need for long-lasting immunosuppression.(10) The development of powerful immunosuppressive agents, such as ciclosporin, has allowed organ transplantation to take a pace of its own.

Ciclosporin is a natural cyclic peptide that inhibits T cell activation initiated by specific antigen. Ciclosporin blocks the activity of calcineurin, a calcium-dependent phosphatase required for a transcription factor, the nuclear factor of activated T cells, to migrate from the cytosol into the nucleus and induce expression of multiple genes, including the T-cell growth factor interleukin 2. In the first randomised trial of ciclosporin, the 11 month survival of cadaveric renal allografts was 73% with ciclosporin alone and 53% with azathioprine and glucocorticoids.(11) Tacrolimus, a macrolide antibiotic that also inhibits calcineurin, was even more effective than ciclosporin in preventing acute rejection of renal allografts, when each of the two drugs was used in combination with azathioprine and glucocorticoids. The rate of acute graft rejection was 26% with tacrolimus, compared with 46% with ciclosporin, and the 1 year graft survival was 82% and 86%, respectively.(12)

Monoclonal antibodies have been developed to target specific molecules on T cells, including the high-affinity interleukin-2 receptor that is expressed on activated T cells. Daclizumab is a genetically engineered human IgG1 monoclonal antibody that binds specifically to the a chain of the interleukin-2 receptor, thereby inhibiting its effects. In a placebo controlled randomised trial, the addition of daclizumab to immunosuppression with ciclosporin, azathioprine, and prednisone decreased the frequency of cadaveric renal graft rejection from 35% to 22%, and improved graft survival at 12 months from 90% to 95%.(13)

Mycophenolic acid selectively and reversibly inhibits inosine monophosphate dehydrogenase, blocking the de novo pathway of purine synthesis. Since lymphocytes rely exclusively on the de novo purine synthesis pathway for the nucleotides necessary for the production of DNA, whereas other cells can also use the salvage pathway, mycophenolic acid selectively affects replication of T and B lymphocytes. Mycophenolate mofetil is a prodrug of mycophenolic acid with potent immunosuppressive activity. A placebo controlled randomised trial showed that 2 g and 3 g mycophenolate mofetil daily for 12 months reduced the rate of acute

rejection of cadaveric renal grafts, when added to ciclosporin and glucocorticoids. At 3 years post-transplantation, mycophenolate mofetil was associated with 7.6% reduction in the frequency of graft loss, indicating that a small but significant effect on graft survival was durable after discontinuation of mycophenolate mofetil.(14)

Rapamycin is a macrolide antibiotic that has a powerful inhibitory effect on interleukin-2 receptor signalling and cell cycle progression of antigen activated T cells. Rapamycin stabilises p27kip1, a cyclin-dependent kinase-2 inhibitor which blocks cell cycle in early G1.(15) In a randomised trial of cadaveric renal allografts, 2 mg and 5 mg rapamycin daily was compared with azathioprine, with each regimen used in combination with ciclosporin and prednisone.(16) The frequency of acute rejection episodes was 17% with rapamycin at 2 mg and 12% at 5 mg, compared with 30% with azathioprine. 1 year graft survival was 97%, 96%, and 98% in the three groups, respectively. In another study, pancreatic islet allografts were sustained in all seven patients for at least 6 months after treatment with rapamycin in combination with tacrolimus and a humanised antibody to the interleukin 2 receptor a chain.(17)

Immunological basis for alloimmune responses

The immunosuppressive drugs developed in the past decade have decreased the rate of acute graft rejection and improved graft survival at 1 year, but patients still require life-long immunosuppression.(18) Understanding of current models for immunity and tolerance might guide a rational use of available drugs and the development of new ones, with the goal of eliminating the need for broad and protracted immunosuppression.

T cells have a critical role in allograft rejection across histocompatibility differences within the same species. T cells express clonally distributed antigen receptors (TCR) that recognise processed antigen fragments as peptides presented by antigen-presenting cells in the groove of MHC molecules. In general, CD4 T cells recognise antigenic peptides presented by class II MHC, and CD8 T cells recognise antigen peptides presented by class I MHC. CD4 and CD8 T cells collaborate in rejection of vascular grafts.(19) Natural killer cells mediate rejection of MHC incompatible haemopoietic stem cell grafts in rodents and, presumably, also in human beings, but they are not involved in rejection of organ grafts. The role of T cells in allograft rejection has been shown by experiments where in vivo T-cell depletion has allowed engraftment of genetically disparate haemopoietic stem cells and skin grafts from the same donor.(3) Furthermore, depletion of T cells from the haemopoietic stem cell grafts prevents graft-versus-host disease.(20) Antigen-presenting cells have an obligatory role in presenting antigenic peptides from the graft and eliciting rejection. Culturing thyroid tissue for several days before transplantation was sufficient to prevent rejection of the thyroid tissue graft.(21) The culture was associated with loss of passenger leucocytes, which would have provided antigen-presenting function in vivo and allowed direct recognition of donor alloantigens by recipient T cells. In other circumstances, however, recipient antigen-presenting cells can take up, process, and present donor alloantigens to recipient T cells, a process called indirect recognition. Graft-versus-host disease was also prevented by eliminating competent antigen-presenting cells from bodies of recipient animals before transplantation of T cell replete marrow grafts from MHC incompatible donors.(22) Thus, graft rejection and graft-versus-host disease stem from cognate interaction of T cells with antigen-presenting cells, which allows recognition of alloantigen and delivery of accessory activation signals. Presentation of alloantigens by incompetent antigen-presenting cells facilitates tolerance.(23)

TCR signalling and activation-induced T-cell death

TCR interaction with antigenic peptide and MHC induces a cascade of signalling events that lead to activation of the various T-cell functions. High avidity or repeated TCR and peptide interactions, however, can produce T-cell exhaustion both in the thymus and the periphery. Mature T cells become susceptible to activation-induced T cell death after their first

encounter with antigen and cell cycle progression promoted by interleukin 2.(24) A second encounter with antigen by activated, cycling T cells induces apoptosis that is mediated by fas and tumor necrosis factor receptors.(25) This mechanism of propriocidal regulation controls the size of the activated peripheral T cells pool during the immune response (figure 1).

High dose antigen stimulation can induce peripheral tolerance by deletion of specific T cells. In a murine model of experimental allergic encephalitis mediated by a T cell clone specific for myelin basic protein, administration of high doses of the specific peptide induced depletion of pathogenic T cells, and prevented the disease.(26) Although there is a strong rationale for treatment of immune disorders with specific peptides, defining the target antigens for allogeneic reactions poses a big challenge. However, antibodies specific for the invariant CD3 domain of the TCR complex induce apoptosis of antigen-activated, cycling T cells.(27) Since activation of resting T cells by antibodies to CD3 requires recruitment and activation of antigen-presenting cells through Fc receptors (FcR), non-FcR-binding antibodies to CD3 do not activate resting T cells but selectively deplete antigen-activated T cells. In a murine model of graft-versus-host disease, a non-FcR-binding antibody to CD3 induced apoptosis of donor T cells activated by recipient alloantigen in vivo and prevented the disease.(28) Selective elimination of antigen-activated T cells by non-FcR-binding antibodies to CD3 could serve as an ideal strategy to prevent allograft rejection and graft-versus-host disease, or to treat autoimmune disorders.

Depletion of alloactivated T cells is a critical mechanism of transplantation tolerance. Treatment with mycophenolate mofetil causes death by apoptosis in a large proportion of activated T cells, suggesting that the immunosuppressive activity of mycophenolate mofetil depends mainly on depletion of alloactivated T cells.(29) By contrast, ciclosporin and tacrolimus block TCR signals, and antibodies to the interleukin-2 receptor a chain block interleukin-2 signals, both of which are required for activation-induced T-cell death (figure 1). Thus, ciclosporin, tacrolimus, and antibodies to the interleukin 2 receptor a chain provide immunosuppression by inhibiting activation signals, but they might also inhibit deletional tolerance.

Two signal model of T-cell activation

Antigen reactive T cells that receive appropriate survival signals, escape activation-induced apoptosis, develop effector function, and establish immunological memory (figure 1). T-cell tolerance, however, can still be induced by non-deletional mechanisms. Stimulation of antigen-specific T cells by antigen-presenting cells that had been modified by gentle fixation resulted in functional inactivation of interleukin-2 gene expression and unresponsiveness to restimulation by normal antigen-presenting cells.(30) This and other experiments suggested that T-cell competence requires stimulation of the TCR plus a second molecule on the surface of T cells that becomes activated by binding to its natural ligand on the surface of antigen-presenting cells. Evidence that CD28 is a pivotal costimulation molecule was provided by experiments in which stimulation of T cell clones with antigen plus an activating antibody to CD28 was sufficient to induce interleukin-2 gene expression.(31) Conversely, stimulation of human T cells by alloantigen in the presence of a blocking antibody to CD28 Fab' fragment caused inactivation of the interleukin-2 gene, which showed that CD28 is crucial for the costimulation of T-cell antigen-specific responses in man.(32) CD28 signalling also promotes T-cell survival by inducing the expression of bcl-xL, a death inhibitor for mature T cells.(33)

The role of CD28 in transplantation was shown in graft -versus-host disease experiments, with mice whose T cells had CD28 deleted by homologous recombination as donors. Recipients of CD28-deficient cells were protected, at least in part, from lethal graft -versus-host disease.(34) CD28 binds to two natural ligands termed B7-1 (CD80) and B7-2

(CD86), that are expressed on activated antigen-presenting cells. A second B7 ligand that is expressed only after T-cell activation is termed cytotoxic T lymphocyte antigen 4 or CTLA4 .(35) The gene encoding CTLA4 is highly homologous with the CD28 gene and is conserved across species. CD28 is expressed constitutively at fairly high density on T cells and binds to B7 with low affinity, whereas CTLA4 which is not expressed on resting T cells becomes expressed at low levels after activation, and has extremely high affinity for B7. A soluble CTLA4 -immunoglobulin fusion protein (CTLA4 -Ig) has been produced for use as a competitive inhibitor for CD28 binding. CTLA4 -Ig therapy blocked human pancreatic islet rejection in mice and induced long-term, donor-specific tolerance.(36) Since then, the effects of CTLA4 -Ig on immune responses and transplantation have been reported in many other papers.

The role of CTLA4 in tolerance

Since the original discovery of CTLA4, many experiments have indicated that CTLA4 is a negative regulator that stops T-cell activation.(37) The cytoplasmic tail of CTLA4 interacts with the shp-2 tyrosine phosphatase which dephosphorylates the TCR complex and terminates T cell activation (figure 2). The definitive evidence that CTLA4 regulates T cell proliferation has come from CTLA4-negative mice.(38) These mice are born normal but all die between 3 and 5 weeks of life with extensive lymphoid proliferation and infiltration of the lungs and other organs. The lack of negative signalling from CTLA4 allows uncontrolled proliferation of T cells which is lethal for the mouse. Thus, a possible problem in the use of CTLA4-Ig is that it would not only inhibit binding of B7 to CD28 and thereby block T-cell activation, but would also inhibit binding of B7 to CTLA4 and block its inhibitory functions. CTLA4 blockade accelerated rejection of cardiac allografts and graft-versus-host disease mortality in CD28-deficient mice transplanted with CD28-deficient t cells.(34,39) Furthermore, in vivo treatment with a CD28-specific antibody was more effective than CTLA4-Ig in preventing graft-versus-host disease mortality after transplantation of wild type allogeneic T cells.(40) Thus, selective CD28 inhibitors are anticipated to be more effective in inducing tolerance than B7 inhibitors.

The role of CD40 ligand in immunity and transplantation

Studies in CD28-deficient animals indicated that mice could survive infections and reject transplants, implying the existence of additional costimulation molecules responsible for T-cell immunity.(41) Activated T cells express a surface receptor designated CD40 ligand (CD40L) because of its ability to bind CD40, which is expressed on the surface of antigen-presenting cells. The function of CD40L, also known as CD154, was originally recognised in patients with congenital mutation of the CD40L gene who suffered from T-cell immunodeficiency and hyper-IgM syndrome.(42) CD40L is required for T cell priming and providing help to cytotoxic T cells.(43-44) T cells from CD40L-deficient mice induced a blunted graft-versus-host-disease and an antibody to CD40L inhibited graft-versus-host disease in mice.(45) Antibodies to CD40L have been shown to delay kidney transplant rejection in primates.(46) The cooperative function of CD40L and CD28 in transplant responses was shown in experiments in which an antibody to CD40L in combination with CTLA4-Ig lengthened skin graft survival in mice.(47) Tolerance to skin grafts, however, was not achieved. In primates, an antibody to CD40L administered in combination with CTLA4-Ig was very effective in sustaining kidney allograft survival.(48) Transplantation of a very high number of haemopoietic stem cells under the cover of an antibody to CD40L and CTLA4-Ig produced sustained chimerism in mice, which became tolerant to skin grafts.(49) These experiments showed that blockade of CD28 and CD40L costimulation molecules on T cells produces additive or synergistic inhibition of allograft rejection.

Tolerance induction by blockade of CD28 and CD40L plus rapamycin

T cells activated by antigen in the presence of CD28 blockade or rapamycin, cannot proceed through the cell cycle and become unresponsive to

subsequent antigen restimulation.(50-51) In primates, tacrolimus proved synergistic with rapamycin in sustaining kidney graft survival, sometimes indefinitely.(52) While the combination of CTLA4-Ig and antibody to CD40L, or rapamycin alone could slightly lengthen survival of skin allografts in rodents, the combination of CTLA4-Ig, antibody to CD40L and rapamycin was able to induce skin graft tolerance.(53) A large fraction of alloreactive T cells was shown to undergo activation-induced cell death when exposed to antigen under costimulation blockade. In these experiments, ciclosporin prevented tolerance to skin grafts by inhibiting TCR signalling required for activation-induced T-cell death.(54) The addition of rapamycin, however, was critical to facilitate tolerance, presumably by inducing unresponsiveness in residual alloreactive T cells. Unresponsive T cells could function as regulatory cells and spread tolerance to other T cells that interact with the same antigen-presenting cells.(55)

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7/7/7 (Item 2 from file: 149)
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Is Islet Transplantation a Realistic Therapy for the Treatment of Type 1 Diabetes in the Near Future?(Edmonton protocol)
Stevens, R. Brian; Matsumoto, Shinichi; Marsh, Christopher L.
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TEXT:

IN BRIEF

Shapiro and colleagues recently reported a 100% cure rate for type 1 diabetes with their "Edmonton protocol" for islet transplantation. This unprecedented success has caused a groundswell of enthusiasm and an unparalleled effort to replicate their experience. It has also raised questions about the clinical reality of this therapy and sparked a dialog about which patients should benefit from receiving this scarce allocated resource. This article reviews the factors contributing to the Edmonton success and obstacles to immediate and long-term expansion of islet transplantation. The authors argue that use of the two-layered method of pancreas preservation will enable the Edmonton protocol to cure diabetes from single and marginal cadaveric donors. A concerted effort will be required to expedite routing of pancreases to islet processing centers and transplant programs. The long-term success and expansion of islet transplantation will depend on not only safer forms of immunosuppression, but also new sources of islet tissue.

Since Banting and Best's discovery of insulin in 1921, the administration of exogenous insulin has been the primary treatment for type 1 diabetes. Although this therapy has enabled millions to survive and lead fairly normal lives, it has fallen short of a cure.

Type 1 diabetes affects millions of individuals and is associated with multiple medical problems, including renal failure and a reduced life expectancy. Tight control of blood glucose levels, achieved with intensive insulin therapy, reduces the secondary complications of diabetes.(1) Unfortunately, such tight control often results in frequent episodes of hypoglycemia.

In contrast to the results achieved with intensive insulin therapy, pancreas transplantation usually results in independence from exogenous insulin, normal glucose levels (both fasting and postprandially), normal Hb(A.sub.1c) levels, and freedom from hypoglycemia.(2) Pancreas transplantation is now widely accepted as a reasonable therapeutic option for many individuals with diabetes and either rapidly progressive or significant end-organ disease (e.g., renal failure) or severe metabolic instability (e.g., hypoglycemic unawareness).(3) Nevertheless, whether one performs a pancreas transplant with systemic or portal venous drainage, both have significant morbidity and mortality rates.(3,4) Thus, most individuals with type 1 diabetes are not candidates for pancreas transplantation.

What has been needed is a less morbid alternative for individuals who have the medical need for a pancreas transplant, but who are not surgical

candidates for this operation. Islet transplantation may now meet this need. This article will review the current state of islet transplantation, with a particular focus on the reasons for the recent success of the Edmonton protocol, and will discuss challenges to the widespread use of islet transplantation in the treatment of type 1 diabetes.

Historical Perspective of Islet Transplantation

Before the Edmonton protocol, human allogeneic islet transplantation was rarely successful. Since the initial observations of Lacy and Kostianovsky in 1967, islet transplantation has reversed hyperglycemia in both large and small animal models of diabetes.(5,6) However, multiple investigators(7-9) have reported early or only partial success with human islet transplantation.

Gores et al.(10) reported two successful instances of islet transplantation (out of six attempts) in type 1 diabetic recipients who received a simultaneous cadaveric kidney transplant. These patients, while euglycemic, had mildly elevated Hb(A.sub.1c) levels.

Although many islet transplant recipients are not insulin-independent, they often have reduced exogenous insulin requirements and decreased Hb(A.sub.1c) levels typically unattainable with intensive insulin therapy.(11) In these patients with reduced insulin requirements, fewer episodes of hypoglycemic unawareness have been noted. Unfortunately, in humans, persistent euglycemia has been the exception rather than the rule.(12)

Recently, reports of greater success in allogeneic islet transplantation have renewed interest in islet transplantation as a possible therapeutic option for some patients with diabetes.(13-15) A close analysis of the successes and failures in both autologous and allogeneic islet transplantation sheds light on the reasons for the unprecedented success of the Edmonton experience.(13)

Autologous Islet Transplantation

A study of autologous islet transplantation foreshadowed the recent success in allogeneic islet transplantation. The low cure rates associated with allogeneic islet transplantation were in stark contrast to the success reported with autologous islet transplantation (8 vs. 75% insulin independence at 1 year).(12) In fact, autologous islet recipients transplanted with (is greater than) 250,000 islets achieved a persistent euglycemic state (70-80%) and normal Hb(A.sub.1c) levels.(16, 17)

With refined islet isolation and purification methods becoming widely applicable, a considerable increase in the number of clinical allogeneic islet transplants has occurred. The 1999 Islet Transplant Registry (ITR) report(12) concluded that establishment of insulin independence after islet transplantation was associated with the following factors: 1) pancreatic preservation times (is less than or equal to) 8 h, 2) islet mass transplanted is adjusted to body weight ((is greater than or equal to) 6,000 islet equivalents per kilogram of body weight (IE/kg)), 3) intrahepatic transplantation, and 4) induction with monoclonal or polyclonal T-cell antibodies.

In the ITR report, one-third of islet allograft recipients with type 1 diabetes who were C-peptide-negative before transplant met all of these characteristics. Forty-eight percent of these patients showed basal C-peptide levels of 0.5 ng/ml; 73% had Hb(A.sub.1c) levels (is less than or equal to) 7%; and 22% were insulin-independent at 1 year's follow-up. Insulin-independent and -dependent recipients did not differ in age, body mass index, duration of diabetes, pre-transplant Hb(A.sub.1c) level or insulin requirements, or age of the cadaveric pancreas donor.

Factors Affecting Allogeneic Islets Transplanted From Cadaveric Donors.

Why have transplanted allogeneic islets--isolated from cadaveric donors--in the past rarely cured patients with diabetes? Cadaveric islets are injured during the procurement, preservation, and isolation process (Table 1).(18, 19) Moreover, islets obtained from cadaveric donors may have increased immunogenicity as compared to noncadaveric islets, potentially

heightening damage to the islets by the recipient's immune system.(20) In addition, immunosuppressive agents, most of which are toxic to islets, must be used in recipients of cadaveric islets.(21) Thus, allogeneic islet grafts likely failed because of the use of toxic immunosuppressive agents and because of immunological mediators of inflammation and antigen-specific immunity.(22-24)

Table 1. Reasons for Islet Allograft Failures in the Past
Organ procurement and preservation methods
* Injury during procurement
* Venous hypertension via UW flush
* Poor cooling of pancreases
Pancreatic processing & islet purification
* Unreliable collagenase activity
* Poor pancreatic distension with collagenase
* Discontinuous ficoll gradient
Islet injury due to immunosuppressive agents
* High-dose steroids
* High-dose calcineurins (cyclosporine or tacrolimus)
Transplantation of inadequate islet mass

Persistent euglycemia after islet transplantation is clearly dependent on factors that affect the viability of the islet preparation. In addition, the inflammatory and immunological challenges the islet graft faces in the post-transplant period significantly affect the success of the transplant.

Toxicity of Immunosuppressants

Tacrolimus (Prograf), cyclosporine (Neoral), and prednisone (Deltasone) are clearly diabetogenic through properties that increase peripheral insulin resistance or by direct islet cell toxicity.(25, 26) These medications are primarily administered orally, which increases portal venous drug concentrations and the possibility of significant injury to intrahepatic islet allografts.

To circumvent this problem, many programs have developed steroid-free and calcineurin-sparing protocols for islet and/or kidney recipients. These protocols are possible with the use of new oral agents, such as mycophenolate mofetil (MMF (Cellcept)) and sirolimus (Rapamune), in combinations that may obviate the need for prednisone.

MMF has been shown to reduce early renal and pancreas rejection rates.(27, 28) Sirolimus is a macrolide antibiotic that binds to the FK binding protein; however, instead of inhibiting interleukin-2 (IL-2) gene expression as does FK506, it blocks intracellular activation signals that block protein synthesis and prevent T-cell progression beyond the G1 phase of the cell cycle.(29)

Although sirolimus is less nephrotoxic than FK506, it is associated with an increased incidence of thrombocytopenia and leukopenia and may be toxic to islets in high doses, also.(30, 31) Recent work by Dr. Breay Paty in Dr. R. Paul Robertson's lab(32) evaluated the toxicity of immunosuppressive drugs on (Beta)-cell function. This study revealed a significant inhibition of insulin secretion in HIT T-15 cells and Wister rat islets by sirolimus, methylprednisolone (Solumedrol), cyclosporine, and tacrolimus. These results confirm the aforementioned hypothesis that low-dose immunosuppression protocols will be especially important in preventing islet toxicity and exhaustion. Nevertheless, sirolimus seems to enhance islet allograft survival without a substantial metabolic impact on islet function.(33, 34)

Therapies designed to deplete or inactivate T-cells may harm islets because they induce the release of cytokines from T-cells. This therapy, termed "induction" when given at the time of transplantation, uses preparations such as antithymocyte globulin (ATG (Thymoglobulin)) or OKT3 (Muromonab). ATG is associated with less cytokine release than OKT3 and with reduced rejection rates in cadaveric renal transplantation.(35, 36) ATG modulates the T-cells for the long term and may account for this beneficial effect on acute rejection rates.(37, 38)

Newer induction agents are available that block the IL-2 receptor pathway by binding to the IL-2 receptor. The two drugs in this new class are humanized murine monoclonal antibody preparations called basilixamab (Simulect) and daclizumab (Zenepax). In clinical trials, these agents have been shown to reduce early rejection rates. Anti-IL-2 receptor antibody therapy may be particularly useful in islet transplantation because it does not induce the release of large amounts of cytokines.(39)

Impact of Cytokine Release on Islet Survival

Cytokines influence multiple cellular processes, from cell maturation to cytotoxicity.(40) In fact, increased cytokine expression is associated with rejection, graft-versus-host disease, and immune-mediated islet injury.(41-43)

Macrophage products (IL-1, IL-6, TNF-(Alpha), and nitric oxide) are primary mediators of transplanted islet dysfunction.(44, 45) During islet cell isolation procedures and subsequent implantation, cytokines (IL-1, IFN-(Alpha) and IFN-(Gamma), and TNF-(Alpha)) are released by passenger leukocytes and Kupffer cells within the liver.(42) Many of these cytokines (IL-1, IFN-(Gamma), IL-6, and TNF-(Alpha)) are deleterious, either directly or indirectly, to islet function and engraftment.(46) In fact, IL-(Beta), IL-6, TNF-(Alpha), and C-reactive protein have been shown to be elevated after intraportal islet transplantation.(47)

Release of TNF-(Alpha) is associated with inflammation and rejection and is toxic to islets.(36, 46, 48, 49) Furthermore, TNF-(Alpha) potentiates the activity of other cytokines with regard to islet cytotoxicity.(42, 50) As mentioned above, standard T-cell cytolytic immunosuppression is associated with a "cytokine storm" phenomenon.

For example, OKT3 induced mRNA expression of several cytokines in human peripheral blood mononuclear cells.(36) In addition, elevated levels of IL-1, IL-2, IL-3, IFN-(Gamma), TNF-(Alpha), IL-6, IL-10, and GM-CSF were observed at various time points after OKT3 administration. For these reasons, blocking TNF-(Alpha) may limit damage to islets, especially when transplanting a low number of islets or when there are donor or isolation factors present that could upregulate TNF-(Alpha) release.

TNFR:Fc (Enbrel) is a recombinant human p75 TNF receptor (dimeric):Fc fusion protein (linked to IgG1). TNFR:Fc binds to and inhibits the bioactivity of TNF-(Alpha) and lymphotoxin (LT) in human and animal studies.(51, 52) Its theoretical benefit is supported by its use in rodent models of islet transplantation.(53)

In a recent study of renal allograft recipients receiving OKT3 for acute rejection,(54) pretreatment with TNFR:Fc lowered the bioactivity of serum TNF-(Alpha) and attenuated the symptoms of OKT3 treatment. At our center, we have utilized TNFR:Fc routinely to block cytokine release and more recently instituted "steroid-free/calcineurin-sparing" immunosuppressive protocols in kidney transplant recipients and have seen a significant reduction in rejection rates ((is less than) 5%).(55) Our early experience supports the safety of these strategies for kidney (and islets) transplants.

Factors Associated With Success of the Edmonton Protocol

The Edmonton protocol addresses many of the barriers discussed above (Table 2).(13) In this trial, pre-uremic diabetic patients were transplanted with allogeneic islets. All achieved a euglycemic state (average follow-up: 11.9 months; range: 4.4-14.9 months).

Table 2. Reasons for the Success of the Edmonton Protocol

Improved organ procurement
* Dedicated procurement team
* Standard procurement procedure
-avoids overperfusion with UW
-pancreas removed before liver and kidneys
-minimal manipulation
Minimized organ preservation injury
* Process pancreas (is less than) 8 h from cross clamp
Improved pancreatic islet cell processing

* Modification of the Ricordi method
* More reliable collagenase solution (liberase)
* Liberase loaded into pancreatic duct using a controlled perfusion technique

Improved pancreatic islet cell purification
* Continuous gradient elutriation (COBE 2991 cell processor)

Reduced immunosuppressive injury to islets
* Elimination of steroids
* Reduction of calcineurin dose
* Sirolimus (minimal islet toxicity)
* Use of IL-2 receptor blocking agent for induction

-Elimination of "cytokine storm"

Transplantation of an adequate islet mass
* Intraportal transplantation of (is greater than) 10,000 IE/kg body weight (multiple donors)

These results were associated with use of a steroid-free and calcineurin-sparing immunosuppression protocol. Induction involved use of IL-2 receptor antibodies. In addition, rapid processing and transplantation of a predetermined minimal islet mass (11,547 (+ or -) 1,604 IE/kg) diminished pancreatic preservation injury. Thus, Shapiro and colleagues have clearly demonstrated that a less "islet toxic" immunosuppression protocol, in conjunction with rapid transplantation of a sufficient islet mass, can result in achievement of a persistent euglycemic state in diabetic recipients.

However, in the Edmonton protocol, for every patient cured, two to three successful isolations from pancreas donors were required. In contrast, pancreas transplantation requires only one donor. If islet cell transplantation could be accomplished using one donor alone, this procedure would surely replace pancreas transplantation. While there are approximately 1 million islets per pancreas, current pancreas preservation and islet isolation techniques enable us to recover fewer than half.(12, 17)

Many simultaneous and sequential steps will need to be taken to expand what has been accomplished from the Edmonton experience and apply it to developing programs (Table 3). Two short-term and critical steps to enhancing this field are: 1) standardizing and expediting the allocation of cadaveric pancreases to either solid-organ pancreas transplant or islet processing centers for transplantation; and 2) improving the preservation of pancreases that may be considered "marginal" to allow for shipping and utilization at these centers, thus expanding the islet donor pool.

Table 3. Requirements for Expanded Application of the Edmonton Protocol (Short-Term)

Improved organ procurement
* Standard method for the pancreas to be used for islets
* Standardize criteria of islet donors among organ procurement organizations (OPOs)
* Develop expedited system of organ placement to islet processing centers

Increased organ preservation time
* Institute two-layer method (TLM) of pancreas preservation (Figure 1A)
* Standardize shipping to processing centers

Improved pancreatic processing
* Decreased variability between liberase lots
* Improved efficiency of Edmonton (Ricordi) method
* Develop assays to predict which pancreases to process

Improved islet purification
* Increased islet/nonislet ratio and variability
* Continuous osmolality and density gradient histidine lactobionate nicotinamide (HLN) and iodixanol

Sponsor regional processing centers linked to multiple OPOs
* Encourage cost-effective management of national resource

* Develop islet banks
* Develop simple standard quality control assays
Multicenter trials with standardized islet preparations
* Reproduce the findings of the Edmonton protocol (in process)
* Transition to single-donor cure protocols
* Develop a consensus on best groups that can benefit
-Compare long-term results of solid-organ pancreas to islet transplantation
* Test new (less toxic, pro-tolerance) immunosuppressive strategies

Improved Pancreas Preservation: the Two-Layer Method

One technique that may help obviate this problem is preservation of pancreases after retrieval from the organ donor but before islet isolation via the two-layer method (TLM) (Figure 1). This technique provides for continued oxygenation of the pancreas by floating the organ on the interface of two fluid layers of differing density. The more-dense fluid layer, perfluorocarbon, carries oxygen in solution. The less-dense layer, University of Wisconsin (UW) preservation solution (Viaspan), is currently used for preservation of solid organs.

(ILLUSTRATION OMITTED)

When pancreases are preserved by the TLM before islet isolation, yields are increased when compared to storage in conventional UW solution in both canine and human 24-h preservation models.(56) Use of the TLM extends the acceptable preservation time of pancreases to 24 h and can approximately double islet yield (Unpublished observations, S. Matsumoto, I. Sweet, C. Marsh, Y. Kuroda, R.B. Stevens).

Adenosine triphosphate (ATP) levels in pancreatic tissue measured after storage using the TLM appear to correlate with eventual islet yields (Figure 1B). Our data suggest that ATP levels measured after TLM preservation may predict low islet yields before processing begins, thus reducing the number of failed pancreas processing events and in turn reducing the total cost of processing pancreases for clinical transplantation.

Islet Transplantation: Current Status

The Edmonton protocol has resulted in unprecedented success in which 100% of patients remain euglycemic. However, this protocol has been applied to a select, relatively healthy group of patients receiving islets from a select group of donors. The Immune Tolerance Network (ITN), supported by the National Institutes of Health's National Institute of Diabetes and Digestive and Kidney Diseases and National Institute of Allergy and Infectious Diseases and by the Juvenile Diabetes Research Foundation International, will attempt to replicate these findings on a national scale.

This widespread implementation of the Edmonton protocol is already driving the standardization of islet processing techniques and will further reveal the toxicity (or lack thereof) of the immunosuppressive strategy. But are the subjects of the ITN effort the most appropriate patients to receive this elaborate and expensive therapy? Is it appropriate to subject healthier diabetic patients to the risks of long-term immunosuppression (nephrotoxicity, infections, malignancy, teratogenicity) in an attempt to prevent end-organ damage from diabetes? Or, should this limited resource be reserved for uremic patients in whom diabetic control is very important,(57) but who have already sustained irreversible damage to some systems?

Typically, patients who are candidates for pancreas or islet transplantation have progressive peripheral complications of diabetes, with proteinuria and/or deteriorating renal function or severe hypoglycemic unawareness. The Edmonton trial was successful in "pre-uremic" patients, but it remains to be seen whether a variation of this strategy can be applied to the most common group transplanted in the International Transplant Registry, namely those requiring a kidney transplant or those who have had a previous kidney transplant.

Clearly, we will need a better understanding of the potential success

rates in these groups, the long-term outcomes, the risk/benefit ratio of this therapy, and how it compares to pancreas transplantation or intensive insulin management before the therapy expands out of clinical trials.

Future Directions

Despite the aforementioned limitations, we are on the verge of a new era in islet transplantation that can be likened to the expansion of liver transplantation in the early 1980s. If the Edmonton trial is replicated on a large-scale basis and the same level of success is found to apply to patients needing a kidney transplant, then the demand for islet transplantation will be exponential. Table 4 outlines the requirements that will be necessary for a major expansion of islet transplantation.

Table 4. Requirements for Islet Transplantation to Become the Preferred Treatment for Type 1 Diabetes

Reduced or eliminated need for immunosuppressive agents
* Induce donor MHC-specific hyporeactivity or tolerance
-co-stimulation blockade
-donor bone marrow-deprived stem cells (co-transplantation)
* Immunological barriers (e.g., encapsulation)
Increased islet masses available for transplantation
* (Beta)-cell expansion
* Xenotransplantation
* Genetically engineered (Beta)-cell lines

Considering the human organ shortage in comparison to the incidence of diabetes, an inexhaustible source of islets will be required. Certainly, we will need islets from either human (Beta)-cell lines generated from progenitor stem cells or other species (xenotransplantation).

Xenotransplantation will most likely require unique protocols to induce tolerance to the foreign tissue or encapsulation of islets to provide a barrier against the allo- or autoimmune attack.(58-60) This is unless, of course, other technologies provide a nontoxic solution, such as gene transfection of insulin-producing genes or the development of microcomputers with real-time biosensing integrated into insulin pumps that provide minute-to-minute control of insulin release for glucose control.(61-63) These topics are beyond the scope of this review, nevertheless we cannot overemphasize the importance of investigating these strategies while at the same time assuring safety to the patient.

To make islet transplantation available for most patients with diabetes, a large source of islets and less toxic immunosuppression strategies (possibly even less toxic than in the Edmonton trial or that employed for kidney transplants) will be required. We envision a safe regimen that induces "partial tolerance" and that can be maintained with minimal immunosuppression.

Tolerance Protocols

Establishing tolerance (specific hyporeactivity of the immune system to donor tissue but not to other foreign antigens) is particularly important in islet transplantation given the direct toxic effect of many immunosuppressants on islets. The following is a brief review of tolerance strategies and their implications for future islet transplantation.

Even before solid-organ transplantation became a clinical reality, Billingham, Brent, and Medawar(64) observed that Freemartin cattle sharing a common placenta displayed red blood cell chimerism . They hypothesized that hematopoetic chimerism may lead to tolerance and acceptance of skin grafts. This idea gained renewed popularity when Starzl and others(65, 66) noted that some patients with long-term functioning liver grafts had circulating dendritic cells at distant sites.

Trials with bone marrow transplantation to augment solid-organ transplants have been performed but with only minimal enhancement of donor-specific hyporeactivity.(67) Despite significant efforts on the part of many researchers, it remains unclear whether strategies aimed toward creating chimerism will promote tolerance and allow for the reduction of chronic immunosuppression.

Although controversial, the tolerizing effect may be derived from the

interaction of antigen-presenting cells (APCs (donor dendritic cells, T-cells, or B-cells)) with donor-reactive T-cells, resulting in apoptosis (direct deletion) or stimulation of regulatory or suppressor T-cells.(68-71) Others speculate that chimerism is a two-edged sword that may promote rejection or tolerance, depending on the antigenic disparities present and the maturational state of the host T-cell. Notwithstanding, many investigators feel that chimerism is not necessary for development of tolerance.(72,73)

Co-Stimulatory Blockade

Briefly, antigens are processed by APCs and presented to host T-cells in context with the major histocompatibility complex (MHC), and this is considered signal one. More recently, CD28 was found to be a pivotal co-stimulatory molecule that provides a necessary "second signal" for the stimulation of T-cells when presented with antigen. Such co-stimulation promotes the production of the cytokine IL-2, a major determinant of the size and tempo of immune responses.(74)

CD28 binds the ligands B7-1 (CD80) or B7-2 (CD86) expressed on activated APCs. T-cells also express a B7 ligand termed cytotoxic T-lymphocyte antigen 4 (CTLA4). Most intriguing have been the recent studies(75, 76) in which CTLA4-Ig therapy, a competitive inhibitor for CD28 binding, blocked human pancreatic islet rejection in mice.

Other studies have revealed that activated T-cells express a surface receptor designated CD40 ligand (CD40L) that binds CD40 expressed on APCs. CD40 ligand is required for T-cell priming and for the development of cytotoxic T-cells. Anti-CD40 ligand antibody therapy has been shown to delay kidney transplant rejection in primates when used alone or in combination with CTLA4-Ig.(77-79)

Can co-stimulatory blockade, which has shown such promise in primate models, work in humans? Unfortunately, despite the success in primate models, early human trials are needed and have yet to be reported.(80)

Interestingly, several investigators have shown that standard immunosuppression agents actually block the immune system's inherent tolerance mechanisms, which are designed to prevent problems such as food allergies. For instance, cyclosporine inhibits cell-mediated clonal deletion, a key mechanism through which the immune system regulates the number of activated T-cells.(81, 82)

Fortuitously, sirolimus, one of the newer immunosuppressive medications, may not block some of these endogenous regulatory mechanisms. Sirolimus inhibits IL-2 receptor signaling and cell cycle progression of antigen-activated T-cells. T-cells activated by antigen in the presence of sirolimus or CD28 blockade cannot proceed through the cell cycle and therefore become unresponsive to antigenic stimulation.(29, 83, 84)

This property of sirolimus and CD28 blockade (provided through induction therapy with anti-thymocyte globulin) might explain why some immunosuppression strategies without steroids are, to date, so successful. Moreover, islet transplants in NOD mice given rapamycin with donor-specific transfusions and co-stimulatory blockade molecules produced long-lasting tolerance.(85, 86)

These clues suggest a regimen to test that could provide "near or partial tolerance" with single- and low-dose maintenance immunosuppression. This strategy, if applied to islet transplantation, should have minimal side effects and cost, making it reasonable for any diabetic patient failing medical management, such as intensive insulin therapy.

Organ Donors and Allocation

Another obstacle to islet transplantation is recognition of this therapy by organ procurement agencies, transplant programs, and the Health Care Financing Administration. Furthermore, there is a limited supply of human cadaveric donors that can potentially be used for pancreas retrieval and islet processing. The UNOS OPTN 2000 annual report reveals that, for 1999, there were 1,627 pancreas donors. There were 2,025 patients waiting for a pancreas transplant, and 1,674 kidney/pancreas transplants were performed in 1995. However, if older or more marginal donors can be used

for islet processing (as compared to the strict criteria typically used for solid-organ pancreas donors), then the donor numbers could expand to equal the number of liver donors, which was 4,954.(87) Herein, the TLM will be vital for using these marginal donors. We anticipate that a national consensus will need to be developed using an algorithm for expedited organ allocation to islet processing centers and financial incentives to induce pancreas procurement for islets.

Implications for Type 1 Diabetes

For most patients and physicians, all of this provides a new thread of hope. Unfortunately, only a few patients will benefit from the ITN or future trials, and many questions have been raised regarding which patients will benefit most from islet transplantation.

Nevertheless, the most exciting point is that this therapy is and must be successful. The efforts of researchers and patients, who have donated money toward research, have made a significant impact. However, the questions of the risks of immunosuppression remain and signal the need for safe tolerance protocols that minimize the risks of long-term immunosuppression.

Conclusion

Islet cell transplantation is a true success, but it is not a panacea for most patients. We propose that the next step for islet transplantation is the implementation of standardized procurement and expedited allocation and shipping systems for pancreases, including using the TLM of preservation, while concurrently refining islet processing techniques. The latter may require not only grants, but also inducements aimed at industry to apply venture capital and research and development efforts in this area. Simul-taneously, we need to forge ahead in clinical trials with different immunosuppressive/tolerance strategies and patient groups to further define success and identity the benefits of this unique benchmark cellular therapy.

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The Future of Organ and Tissue Transplantation: Can T-Cell Costimulatory Pathway Modifiers Revolutionize the Prevention of Graft Rejection?
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Grand Rounds at the Clinical Center of the National Institutes of Health Section Editors: John I. Gallin, MD, the Clinical Center of the National Institutes of Health, Bethesda, Md: David S. Cooper, MD, Contributing Editor, JAMA.

Transplantation therapies have revolutionized care for patients with endstage organ (kidney, liver, heart, lung, and pancreatic (beta)-cell) failure, yet significant problems persist with treatments designed to prevent graft rejection. Antirejection therapies are not always effective, must be taken daily, and are both expensive and associated with well-known toxic effects. Recent advances have suggested that the immune system has more selfregulatory capability than previously appreciated. In this review, we discuss immune system function and new therapeutic agents that modify so-called costimulatory receptor signaling to support transplant function without generally suppressing the immune system.

Can T-Cell Costimulatory Pathway Modifiers Revolutionize the Prevention of Graft Rejection?

CURATIVE TRANSPLANTATION has become a widely accepted and utilized treatment modality. In 1997 alone, nearly 20 000 Americans received therapeutic and often lifesaving organ transplants.(1) Unfortunately, curative transplantation is made available to only a fraction of those who need it. United Network for Organ Sharing data indicate that patients in need of organ transplants outnumber available organs 3 to 1. Even that statistic minimizes the health benefit that could be achieved were organ and tissue transplantation more widely available. An estimated 75% of annual US mortality is caused by chronic illnesses (eg, cardiovascular and cerebrovascular diseases, chronic obstructive pulmonary disease, chronic liver disease and cirrhosis, malignant neoplasms, and diabetes) that would benefit from more widely available transplant therapies.(2)

Several factors, aside from the inadequate organ supply, limit more widespread transplantation. Despite great improvements in immunosuppressive therapies to prevent acute rejection, chronic graft rejection remains a significant problem. For example, half of renal allografts fail within 10 years of transplantation.(3,4) Indeed, the majority of kidney grafts that fail after the first year are lost to chronic graft rejection.(5) Unfortunately, while immunologists understand the mechanisms underlying acute graft rejection in some detail, chronic rejection is less well understood and probably represents several different processes (immunological and nonimmunological) that converge to negatively affect allograft function.(6) Because the primary histological feature of chronic

graft rejection is a proliferative vasculopathy, several investigators have explored the role growth factors may play in the process.(7) Important factors also shown to influence the incidence of chronic graft rejection include the number of acute rejection episodes that have occurred, donor organ quality, the use of certain immunosuppressive drugs, and more classic risk factors for vascular disease, such as elevated blood pressure and serum lipid levels.(5)

In any case, the shortcomings of current therapies have prevented lifesaving skin allografts for serious burn victims, curative pancreatic islet cell transplants for patients with type 1 diabetes, (8-10) and small bowel transplants for patients with short-bowel syndromes.(11) Even when current immunosuppressive therapy is effective, as it is in most patients receiving solid organ transplants today, the therapy is costly,(12) usually must be taken daily for life, and is associated with a significantly increased risk for (depending on the specific agent used) infection, cancer, and other morbidity, including osteoporosis, hyperglycemia, cataracts, and renal dysfunction. (13-18) Transplant recipients also must comply with a fairly complex daily drug-dosing schedule, and they must be followed up closely to minimize the risk of known complications while they strike a balance between too much or too little immunosuppression. There is a real need for improved ways to maintain allograft function by preventing immune system-mediated rejection.

Immunology Overview

All multicellular organisms have systems that defend against invasion by other biological organisms. Even a sponge will destroy tissues grafted from an unrelated sponge. (19,20) Thus, it is safe to surmise that multicellular organisms without immune systems do not survive long under evolutionary pressure, since none are known to exist today. Transplantation of tissues or organs from one member of a species to another is not a likely natural event, however, so the immune response to an allograft is a by-product of the response to more immediate threats to the survival of a species.

Evolutionary immunologists have subdivided the immune system into 2 broad and interrelated systems, the innate and adaptive immune systems. (21) The innate system, the more primitive of the 2, consists of phagocytic cells that use a variety of clues to recognize, engulf, and digest invading pathogens. In mammalian species, the innate immune system consists of polymorphonuclear cells, monocytes, macrophages, and dendritic cells. The adaptive immune system first appeared some 400 million to 600 million years ago along with vertebrate evolution, and a more advanced system, with definable T and Blymhocyte subsets, evolved with the common predecessor for all mammalian and avian species some 200 million to 300 million years ago. The defining characteristic of this advanced system is the expression, on the surface of the adaptive immune system cells (T and Blymphocytes), of antigen-specific receptors designed to recognize only 1 target. B lymphocytes make antigen-specific antibodies, and T lymphocytes are responsib le for what is called the cell-mediated immune response. It is this cellular immune response that is primarily responsible for the rejection of allografted tissues and organs.

T Lymphocytes Direct the Antigraft Immune Response

The T lymphocyte is primarily responsible for directing the immune response that recognizes and then destroys cells damaged by toxins or viruses. The T lymphocyte also directs the immune response against cells, tissues, or organs transplanted from another member of the same species. Each adult human has approximately 10 (10) to 10 (12) T lymphocytes, any 1 of which can recognize only 1 target. But since most specific lymphocytes exist in multiple copies, it is estimated that a normal human's T lymphocyte repertoire can recognize approximately 10 (9) to 10 (10) different antigens. (22)

T lymphocytes, however, do not recognize whole antigens. Rather, antigen specific T-cell receptors (TCRs) recognize small peptide fragments of whole antigens presented by the major histo compatibility complex (MHC)

proteins present on cell surfaces (FIGURE 1). Class I MHC molecules, present on nearly all tissue cells, present fragments of proteins made within the cell (eg, both self-proteins and proteins that may be encoded by infecting virus genes). Class II MHC molecules, found only on specialized antigen-presenting cells, present fragments of antigens made outside the cells, which are then phagocytosed, digested, and finally presented by the specialized, antigen-presenting cell. The MHC molecules themselves are polymorphic, so it is highly unlikely that 2 unrelated individuals will have identical MHC types. Indeed, this donor-host MHC disparity is primarily responsible for triggering the antigraft immune response and is the reason that measures are taken to scan the list of potential recipients for an MHC match whenever a donor organ becomes available. Finding a complete match greatly decreases the likelihood of a vigorous immune response against the transplanted tissue. (4)

T lymphocytes are often categorized into 2 subgroups based upon the presence of other protein complexes on their surface, the so-called CD4+ and CD8 T-cell subsets (Figure 1). CD4+ T cells recognize antigens presented by the MHC class II complexes only on professional antigen-presenting cells (cells of the innate immune system), while CD8+ T cells recognize antigens presented by MHC class I complexes. Both CD4+ and CD8+ T cells play important roles in the immune response that results in graft rejection. Moreover, both the innate and the adaptive immune systems discussed above have important roles in the antiallograft immune response.

Approximately 1% of an individual's T cells are cross-reactive for another (unmatched) individual's MHC complex proteins. Thus, approximately 1% of a recipient's T cells might directly recognize MHC molecules present on transplanted tissue cells. In addition, when the host's innate immune system cells encounter damaged tissue or cells within the transplant (from surgical or ischemic injury), those damaged donor cells are ingested and antigens from them are presented to the host T cells (indirect recognition), further promoting the antigraft immune response.

The 2-Signal Model for T-Cell Activation

While TCRs must recognize MHC-presented antigen peptides to activate an antigen-specific T cell, Lafferty and Cunningham (23) suggested that recognition alone was not sufficient to activate a T cell. Their study expanded on earlier work by Bretscher and Cohn (24,25) and resulted in the development of the 2-signal model for T-cell activation (FIGURE 2). The model proposed that TCR recognition of an appropriately presented antigen would deliver a signal 1 to the T cell, but that a simultaneously delivered signal 2 (the costimulatory signal) was required to activate the T cell. Two important corollaries resulted from this hypothesis. First, a signal 2 delivered without antigen recognition was a neutral event for the T cell. Second, if a T cell encountered its cognate MHC antigen but did not receive a signal 2 at the time of the antigen recognition, that T cell would either die or be rendered resistant to activation in future encounters with that antigen.

The second corollary suggested a novel way to prevent an antiallograft immune response after therapeutic transplantation. The other typical antirejection regimens used therapeutically to date have resulted in nonspecifically blunted T-cell responses. For instance, glucocorticoids and calcineurin phosphatase inhibitors (cyclosporine and tacrolimus) are now known to impair TCR-mediated signaling (ie, signal 1) to the T cell. Other agents in wide use, such as azathioprine and mycophenolate mofetil, interfere with purine synthesis and therefore impede all rapidly proliferating cells, such as T cells responding to an antigen challenge. The most nonselective tools were those that indiscriminately depleted T cells, eg, hemi-body irradiation, thoracic duct drainage, and agents such as antithymocyte globulin. (4) The 2-signal model suggested that an agent that specifically interrupted the proposed T-cell costimulatory receptor when the T cells first encountered the transplanted organ could inactivate only those antigen-specific T cells, leaving unimpaired other T cells that did not encounter their cognate antigens.

Costimulatory Molecules

June and colleagues (26) found that stimulating T cells with a mixture of 2 antibodies, 1 that stimulated the TCR and another directed against a previously uncharacterized 44-kd cell surface receptor, induced rapid T-cell proliferation that, unlike all other ways of activating T cells via surface receptor antibodies, was not inhibited with cyclosporine. (27) The cell surface receptor identified by that antibody, originally called Tp44 and later named CD28, was the first of several costimulatory T-cell receptors to be reported and extensively studied. While costimulatory receptor-like function has since been reported for several receptor--counter-receptor pairs, the 2 shown in FIGURE 3, A and B, have been of particular interest to transplant immunologists because of their ability to prevent allograft rejection in experimental animal models.

The CD28-B7 counter-receptor group consists of 4 unique receptors. Two B7 receptors, B7-1 (or CD80) and B7-2 (or CD86), have been cloned (28-30) and are known to be expressed by activated antigen-presenting cells, albeit with slightly different kinetics and levels of expression. The interaction of either B7 with a T cell's CD28 can costimulate T-cell activation if a TCR-generated signal is delivered simultaneously. (31) T cells also express another receptor called CTLA4 (or CD152) that also serves as a counter-receptor for both B7 receptors. (32,33) While the functional relevance of CD152 was debated for several years after its identification, 2 simultaneous reports (34,35) describing the phenotype of CD152 knockout mice cleared up much of the confusion. Both articles reported that mice with disrupted CD152 genes died prematurely, showing evidence of profound lymphocyte activation manifested by massive splenomegaly, lymphadenopathy, and lymphocytic infiltration of the heart, pancreas, and other parenchymal t issues. Thus, while many of the details remain obscure, CD28-B7 interactions stimulate T-cell activation, while CD152-B7 interactions seem to restrain T-cell responses.

Linsley et a1 (33) sought to develop a reagent that would block the CD28-B7 interaction. Using molecular biological techniques, they created chimeric molecules, composed of the extracellular domain of either CD28 or CD152 coupled with the IgG heavy chain (CD28-Ig and CTLA4-Ig, respectively). CD28-Ig was subsequently found to ineffectively interfere with CD28-B7 signaling, but CTIA4-Ig (36) appeared to compete effectively with CD28 for binding to both B7 ligands and therefore to prevent T-cell activation.

The CD40-CD154 receptor pair (Figure 3, B) has an even more recent history in transplantation immunology. B lymphocytes express CD40, a cell surface receptor known to be important in mediating immunoglobulin class switching from the early IgM response to the later IgG response following antigen stimulation. The study of hyper-IgM syndrome determined that such patients had a defect in the T-cell counter-receptor for the B cell's CD40. (37) This T-cell counter-receptor was originally called gp39, then CD40 ligand (or CD40L), and, most recently, CD154. Thus, while CD154 was identified because B-lymphocyte function is defective when it is genetically absent, dramatic anti-CD 154 antibody effects on T-cell function were subsequently identified.

Costimulatory Receptor Blockade in Small Animal Transplant Models

Interest in T-cell costimulatory receptor signaling greatly increased when Lenschow et al (38) reported a landmark study demonstrating that costimulatory receptor blockade could specifically prevent graft rejection in mice. Diabetes was induced in mice by administering a pancreatic (beta)-cell toxin (streptozotocin), and the mice were then transplanted with human pancreatic islets injected under 1 kidney capsule. The mice were temporarily cured of diabetes , but the human islets were rejected a few days later, and disease recurred. Lenshow et al demonstrated that CTLA4 -Ig administered during the 2 weeks immediately after the transplantation allowed for long-term graft function and diabetes -free survival. They also reported that if the kidney containing the human islets was excised several weeks after the transplant ,

diabetes promptly recurred, demonstrating that the transplanted human islets were responsible for maintaining euglycemia. Islets injected under the animal's contralateral kidney capsule following t he surgical removal of the initially transplanted islets could permanently cure diabetes only if the islets were from the original human donor; islets harvested from another human donor were rejected. It appeared that the mouse immune system had learned to recognize tissues from the original human donor while responding normally to similar cells (islets) from a different human donor. Other reports followed, demonstrating that allograft survival in rodents could be induced reproducibly by agents interfering with CD28-B7 signaling. (39-42) More recent reports have suggested that such agents could even abrogate the vasculopathy characteristic of chronic rejection. (43-46)

The first reports suggesting that agents modulating the CD40-CD 154 interaction could similarly induce long-term graft survival first appeared in 1995. Parker et a1 (47) found that donor-specific small lymphocytes and anti-CD154 given before and for 7 weeks after the transplant of allogeneic islets under a kidney capsule permanently prevented graft rejection and relapse of diabetes . Moreover, they proposed that the mechanism of graft acceptance was similar to that induced by CTLA4 -Lg, (48). that is, small lymphocytes are known to express little or no BY. Furthermore, the interaction of CD40 on small B lymphocytes with CD 154 present on activated T cells is known to lead to the up-regulated expression of B7 on B lymphocytes. Rossini and colleagues (48) proposed that the anti-CD154 had prevented B7 expression from being up-regulated, allowing the MHC molecules present on the donor small lymphocytes to interact with the host's anti-donor-specific T cells without receiving the required B7-CD28 costimulatory signa 1, thus making the host tolerant to the donor tissues. Several other reports documenting the graft -sparing effect of antiCD154 have since appeared. (48-53)

While these rodent studies generated considerable interest, transplant immunology literature is filled with promising therapies developed in mouse transplant models that have failed in other species. Indeed, using conditions similar to those that resulted in great success in rodents, we (54) and others (55) found that CTLA4-Ig did not reliably prevent allograft rejection in nonhuman primates. Regardless of the relative ease with which graft acceptance can be induced in mice, no simple and nontoxic regimen had ever been reported to prevent the rejection of full-thickness allogeneic skin grafts, even in mice, prior to a study reported by Larsen et al. (56) Reasoning that anti-CD 154 prevented the expression of BY from being up-regulated, and CTLA4-lg bound to B7 and prevented B7-CD28 signaling (FIGURE 4), Larsen and colleagues tested the effect of both agents in combination using a mouse full-thickness skin allograft model. Remarkably, while neither agent alone was effective, a short course of both agents comb ined allowed long-term skin allograft survival in nearly all experimental animals tested. Equally surprising was their observation that the graft-promoting effect of CTLA4-Ig plus anti-CD 154 was abrogated if the agents were coadministered with cyclosporine. Because cyclosporine interferes with the signal transduced by the antigenengaged TCR (signal 1 in the 2-signal model), these data strongly suggested that the costimulatory pathway--modifying reagents were not simply another class of immunosuppressive drugs. Indeed, the agents appeared to require TCR-mediated antigen recognition for their full therapeutic effect.

Costimulatory Pathway Modifying Reagents in Nonhuman Primate Models

Upon learning of the efficacy achieved using combination CTLA4 -Lg plus antiCD154 therapy in the murine full-thickness skin allograft model, we tested a similar regimen in a rhesus monkey renal allograft model. (54) Adolescent rhesus monkeys underwent bilateral nephrectomy and then were given purposely mismatched renal allografts. All the control allografts were rejected within 8 days, as expected. The animals treated with a combination of CTLA4 -Ig plus anti-CD154 for 28 days posttransplant all

maintained normal graft function for at least 6 months. In contrast to the results reported in the mouse transplant models, however, anti-CD154 alone was effective at preventing acute renal allograft rejection. Consistent with the rodent studies, the costimulatory pathway-modifying reagents were well tolerated. The animals' hematologic parameters were not affected, they did not display any evidence of susceptibility to infectious agents, their wounds healed normally, and they gamed weight consistent with colony mates. S ince those studies were published, we have conducted others to determine the minimally effective dose of the costimulatory pathway-modifying reagents, whether the agents can maintain their effectiveness when administered with traditional graft -sparing agents, and tests to further delineate the mechanism underlying the agents' graft -sparing effect. Anti-CD154 monotherapy is highly effective at preventing acute renal allograft rejection. (57) For instance, 2 animals given anti-CD154 in 6 doses over the first month after their kidney transplantations demonstrated impressive graft survival; i animal rejected the kidney 120 days posttransplantation, and the other continues to display normal graft function more than 750 days after surgery (ie, no antirejection therapy for more than 700 days). Nine additional animals that received a kidney allograft, 6 anti-CD154 doses over the first month, and monthly doses thereafter for 5 subsequent months did not reject their grafts . However, consistent with the mouse data that preceded our primate studies, traditional immunosuppressive agents appeared to abrogate antiCD154 effectiveness. The outcome in animals treated with tacrolimus, steroids, or mycophenolate mofetil was not uniformly successful. (57) Most intriguing, allograft biopsies revealed that T cells that appeared to be activated infiltrated the transplanted tissue, yet those cells did not invade the kidney blood vessels, tubules, or glomeruli. (37) Moreover, we have collaborated with other investigators to test whether the reagents can support the survival of islet allografts transplanted into rhesus monkeys that underwent pancreatectomy . Those experiments have yielded similar results.58

How Do the Reagents Work?

The immunological background of these developments in transplantation immunology have recendly been reviewed in greater detail elsewhere. (59) The simple model shown in Figure 4, as first proposed by Lenschow et al (38) and Rossini et a1 (48) to explain the ability of costimulatory pathway--modifying reagents to prevent the rejection of transplanted tissues and organs, is now known to be oversimplified. (60) For instance, the grafts of animals given costimulatory pathway-modifying reagents to prevent transplant rejection express B7 molecules to a degree that is indistinguishable from that seen in organs undergoing rejection in untreated animals. (56)

It is currently believed that the interaction between T cells (of both the CD4 and CD8 subsets) and cells of the innate immune system underlies the immune response that leads to the rejection of a transplanted organ (FIGURE 5). Donor tissue antigens are released as a result of the tissue damage that invariably results during harvest and/or transplantation, and the host's innate immune system cells, including dendritic cells, pick up those foreign antigens. In addition, since inflammatory cytokines are locally released as a result of simple surgical trauma, local dendritic cells in the area of the transplant are partially activated. Resting dendritic cells express low levels of MHC class II and B7 costimulatory ligands, and they do not express cytokines such as interleukin (IL) 12. But when dendritic cells are activated, they produce more IL-12 and more highly express MHC class II and B7 molecules. If 1 of these partially activated dendritic cells presenting a fragment of the foreign MHC encounters a CD4 T ce ll with a TCR specific for that MHC antigen, that antigen-specific T cell will become activated.

While the resting T cell expresses little or no CD154, the activated CD4 T cell rapidly increases its CD154 expression. At this point, a positively reinforcing cycle is established. The CD4 T cell's CD154 serves

as a ligand for the dendritic cell's, CD40, which, in turn, leads to further activation of the dendritic cell. Fully activated dendritic cells highly express several gene products (eg, MHC class I and II molecules, B7 costimulatory molecules, and adhesion molecules) and produce more inflammatory cytokines such as IL-12. Based on studies by Ridge et al, (61) Schoenberger et al, (62) and Bennett et al, (63) Lanzavecchia described this fully primed dendritic cell as one that can pass along a "license to kill," (64) in that it can effectively stimulate the maturation of CD8 T cells called cytotoxic T lymphocytes, (GIL) known to be required for full antigraft immune responses (Figure 5).

Given this proposed sequence of events, anti-CD 154 could interrupt the escalating sequence of activation at the outset by blocking the CD4 T cell from further activating the partially activated dendritic cell, thus preventing the maturation of CTLs. Even this model is oversimplified, however. For example, anti-CD154 can appear to interrupt an ongoing rejection episode in primates. (54) Other studies have reported that anti-CD 154 can prevent ongoing autoimmune syndromes in laboratory animals, even late in the disease process. (65,66) Furthermore, the model suggests only an indirect role for anti-CD154, that of blocking CD4 T cells from fully activating dendritic cells. However, we have identified some evidence for a direct effect of anti-CD154 on inactive CD4 T cells (P.J. Blair, PhD, unpublished data, 1999).

The Future of Transplantation?

Advances in immunosuppressive therapy have resulted in organ transplants that have protected thousands of patients from end-stage disease. However, the immunosuppression paradigm responsible for many of the gains made to date may have reached the limit of usefulness. Newer immunosuppression regimens have led' to proportional increases in posttransplant morbidity, with little additional improvement in graft or patient survival. (67,68) The goal of current clinical research in transplantation must shift toward developing antirejection therapies that have long-lasting efficacy and rely less on global immunosuppression. Research in T-cell costimulation has disclosed the previously underappreciated ability of the immune system to finely regulate its activity. By exploiting these regulatory mechanisms, durable rejection-free allograft survival might be feasible using intermittently dosed, relatively nontoxic agents. Clinical trials rigorously investigating these new agents are warranted, and their findings will be anticipated with great interest.

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7/7/9 (Item 4 from file: 149)
DIALOG(R)File 149:TGG Health&Wellness DB(SM)

01845078 SUPPLIER NUMBER: 55124460 (THIS IS THE FULL TEXT)
Long-Term Survival and Function of Intrahepatic Islet Allografts in Baboons Treated With Humanized Anti-CD 154.
Kenyon, Norma S.; Fernandez, Luis A.; Lehmann, Roger; Masetti, Michele; Ranuncoli, Alessandra; Chatzipetrou, Maria; Iaria, Giuseppe; Han, Dongmei; Wagner, Joseph L.; Ruiz, Philip; Berho, Mariana; Inverardi, Luca; Alejandro, Rodolfo; Mintz, Daniel H.; Kirk, Allan D.; Harlan, David M.; Burkly, Linda C.; Ricordi, Camillo
Diabetes, 48, 7, 1473
July,
1999

TEXT:

Clinical islet cell transplantation has resulted in insulin independence in a limited number of cases. Rejection, recurrence of autoimmunity, and impairment of normal islet function by conventional immunosuppressive drugs, e.g., steroids, tacrolimus, and cyclosporin A, may all contribute to islet allograft loss. Furthermore, intraportal infusion of allogeneic islets results in the activation of intrahepatic macrophages and endothelial cells, followed by production of proinflammatory mediators that can contribute to islet primary nonfunction. We reasoned that the beneficial effects of anti-CD154 treatment on autoimmunity, alloreactivity, and proinflammatory events mediated by macrophages and endothelial cells made it an ideal agent for the prevention of islet allograft failure. In this study, a nonhuman primate model (Papio hamadryas) was used to assess the effect of humanized anti-CD154 (hu5c8) on allogeneic islet engraftment and function. Nonimmunosuppressed and tacrolimus-treated recipients were insulin independent posttransplant, but rejected their islet allografts in 8 days. Engraftment and insulin independence were achieved in seven of seven baboon recipients of anti-CD154 induction therapy administered on days -1, 3, and 10 relative to the islet transplant. Three of three baboons treated with 20 mg/kg anti-CD154 induction therapy experienced delayed rejection episodes, first detected by elevations in postprandial blood glucose levels, on postoperative day (POD) 31 for one and on POD 58 for the other two. Re-treatment with three doses of anti-CD154 resulted in reversal of rejection in all three animals and in a return to normoglycemia and insulin independence in two of three baboons. It was possible to reverse multiple episodes of rejection with this approach. A loss of functional islet mass, as detected by reduced first-phase insulin release in response to intravenous glucose tolerance testing, was observed after each episode of rejection. One of two baboons treated with 10 mg/kg induction therapy became insulin independent posttransplant but rejected the islet graft on POD 10; the other animal experienced a reversible rejection episode on POD 58 and remained insulin independent and normoglycemic until POD 264. Two additional baboon recipients of allogeneic islets and donor bone marrow (infused on PODs 5 and 11) were treated with induction therapy (PODs -1, 3, 10), followed by initiation of monthly maintenance therapy (for a period of 6 months) on POD 28. Rejection-free graft survival and insulin independence was maintained for 114 and 238 days, with preservation of functional islet mass observed in the absence of rejection. Prevention and reversal of rejection, in the absence of the deleterious effects associated with the

use of conventional immunosuppressive drugs, make anti-CD154 a unique agent for further study in islet cell transplantation. Diabetes 48:1473-1481, 1999

The requirement for and incomplete success with chronic generalized immunosuppression has limited the application of curative islet cell transplantation for type i diabetes. Moreover, conventional immunosuppressive drugs, such as steroids, cyclosporin A, and tacrolimus, are known to impair normal islet function (1), increase susceptibility to infection and malignancy, stunt normal growth and development, and result in direct organ toxicity (2). Nevertheless, human islet cell allotransplantation in patients with type i diabetes has resulted in normoglycemia and normalization of other metabolic parameters, in the absence of hypoglycemia, in a limited number of patients who have received standard immunosuppression (3-9). Graft function of (is greater than) 6 years' duration resulting in improved glycemic control has now been documented for two diabetic recipients of allogeneic islets (9).

The limited success in human islet allotransplantation is probably multifactorial. Factors limiting success include the diabetogenic effect of immunosuppressive drugs, recurrent anti-(Beta)-cell autoimmunity, and/or nonspecific inflammatory events that occur when islets are transplanted into the hepatic microenvironment. Over the years, while several approaches have proven successful for engraftment and long-term graft survival in rodent islet cell transplantation models, these approaches have not translated uniformly or consistently to either canine or nonhuman primate preclinical models or to humans.

Recently, modulation of the immune response, via blockade of the CD40-CD154 costimulatory pathway (10-12), has been reported to result in long-term renal allograft survival in nonhuman primates (13). CD40 is expressed on antigen presenting cells (APC), including B-cells, dendritic cells, macrophages, and endothelial cells. CD154, also known as CD40 ligand (CD40L), is rapidly and transiently upregulated on (CD4.sup.+) T-cells upon interaction of the T-cell receptor with the antigen/major histocompatibility complex on APC. The subsequent interaction of CD40 on APC with CD154 on the T-cell has multiple effects, including upregulation of the CD80 and CD86 costimulatory molecules and activation of the signal cascade that leads to generation of an allospecific immune response. Blockade of this interaction prevents the generation of cytotoxic effector cells and T-dependent antibodies (10-12). In addition, the CD40-CD154 pathway is critical for the generation of proinflammatory cytokines and nitric oxide by activated macrophages and endothelial cells (14-18).

In murine models, blockade of the CD40-CD154 pathway, via treatment with monoclonal antibody specific for CD154, has been shown to prevent the rejection of allografts (19-24), ameliorate autoimmune disease (25-34), and prevent the occurrence of acute and chronic graft versus host disease (35-37). Even more striking is the aforementioned demonstration of engraftment and long-term survival of allogeneic kidneys in a rhesus monkey model (13), achieved via monotherapy with a humanized anti-CD154 specific monoclonal antibody, 5c8 (Biogen, Cambridge, MA) (38).

This study was designed to assess the efficacy of humanized anti-CD154 (hu5c8) in a nonhuman primate model of islet allotransplantation. The results obtained clearly demonstrate allogeneic islet engraftment and insulin independence, with long-term function (up to 300 days) for all baboons treated with adequate doses of anti-CD154 (hu5c8). Furthermore, we have demonstrated that it is possible to halt the progress of islet allograft rejection and return the animals to normoglycemia and insulin independence by treating the recipients with additional doses of anti-CD 154 (hu5c8).

RESEARCH DESIGN AND METHODS

Baboons. Baboon (Papio hamadryas) donors were obtained from the Southwest Foundation (Alice, TX) and recipients from the Mannheimer Foundation (Homestead, FL). Donors ranged from 4 to 10 years of age and recipients from 0.5 to 2 years of age; both males and females were used.

Recipients were pair-housed and fed twice daily. The experiments described in this study were conducted according to the principles set forth by the Institute of Laboratory Animal Resources, National Research Council (38a).

Identification of donor-recipient pairs. Alloreactive donor-recipient pairs were chosen based on positive mixed leukocyte culture (MLC) reactivity, with stimulation indices of (is greater than or equal to) 11.3. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized peripheral blood via density gradient centrifugation over ficoll-paque (Pharmacia, Piscataway, NJ). In a one-way MLC, (10.sup.5) recipient PBMC were used as responders against (10.sup.5) (Gamma)-irradiated (3,000 rad) donor PBMC. Cultures were established in 96-well U-bottom tissue culture clusters. The medium consisted of, RPMI-1640 (Gibco BRL, Grand Island, NY) supplemented with 100 U/ml penicillin, 100 (micro)g/ml streptomycin, 2 mmol/1 L-glutamine, 1x vitamins, 0.1 mmol/1 nonessential amino acids, 1 mmol/1 sodium pyruvate, and 10 mmol/1 HEPES buffer (all Gibco BRL). Cultures were incubated for 5 days in an atmosphere of 5% C(O.sub.2) in air at 37 (degrees) C, pulsed with 1 (micro)Ci of tritiated thymidine (((sup.3)H)thymidine), incubated overnight, and harvested. Counts per minute of incorporated (sup.3H)thymidine were determined, and data were expressed as counts/min or as stimulation index (counts per minute from experimental cultures of recipient PBMC versus irradiated donor cells divided by counts/min from control cultures of recipient PBMC versus irradiated autologous cells). Results of preliminary in vitro experiments revealed that use of MLC medium supplemented with human serum (Sigma, St. Louis, MO) resulted in low background and high specific reactivity (compared with medium containing fetal calf or normal baboon serum). The range of MLC stimulation indices for donor-recipient pairs used in this study was 11.3-40.8, reflecting a high degree of donor-recipient alloreactivity.

Donor pancreatectomy and islet isolation. The technique used for donor pancreatectomy is as follows. First, the splenocolic and splenorenal ligaments were divided so that the spleen, together with the tail of the pancreas, was mobilized. After the performance of a Kocher maneuver, the head of the pancreas was dissected from the second portion of the duodenum. The common bile duct, the main (Wirsung), and occasionally, the secondary (Santorini) pancreatic ducts were ligated and divided. A 14-gauge catheter was placed in the infrarenal aorta, and the animal was exsanguinated (the blood was collected to obtain donor serum and peripheral blood leukocytes). After exsanguination, the gastrosplenic ligament was divided and sharp dissection was performed between the stomach and the pancreas. The splenic and pancreaticoduodenal vessels were divided, and the pancreas was taken out, en block, with the spleen. The mesenteric lymph nodes were also collected. The pancreas, spleen, and lymph nodes were placed in Hanks balanced salt solution for transportation to the lab. The spleen and nodes were processed and cryopreserved to serve as a source of donor cells posttransplant. Cold ischemia time averaged 0.5 (+ or -) 0.1 h. Baboon islet isolation was undertaken via minor modifications of the automated method for human islet isolation (39,40) using Liberase (Boehringer Mannheim, Indianapolis, IN) at a concentration of 0.47 mg/ml. A three-layer discontinuous Euroficoll gradient (densities: 1.108,1.097, 1.037) was used for purification of islets from the pancreatic digest (41). The tissue was bottom-loaded with the 1.108 layer and centrifuged in a COBE 2991 blood cell processor (COBE, Lakewood, CO) as previously described. The number, volume, and purity of islets were determined as follows. The final islet preparation was suspended in 250 ml RPMI 1640 solution, and three 100-pi samples were stained with dithizone (42) and then counted to assess total islet yield. These data were mathematically converted (43) to the total number of islets with an average diameter of 150 (micro)m (islet equivalent (IEQ)). Islets were cultured overnight at 25 (degrees)C, 5% C(O.sub.2).

Recipient pancreatectomy and intrahepatic islet cell transplantation. The anesthesia and mechanical cleansing of the recipient was similar to that undertaken for the donor. Hemodynamic parameters, respiration,

temperature, and arterial hemoglobin oxygen saturation (noninvasive measurement via pulse oximetry) were monitored. A 24-gauge intravenous catheter was placed for administration of fluids and medications. The technique used for total pancreatectomy has been previously described by Ericzon et al. (44) for a cynomolgous monkey model.

Cultured islets were washed and resuspended in 20 ml of medium containing 10% donor serum and 20 mg of hu5c8. The range of IEQ transplanted per kilogram of recipient body weight was 12,549-27,429 (pellet volume 0.2-0.4 ml, purity (is greater than) 95%). An adequate number of viable islets was obtained from one donor to allow for transplantation of two recipients. Over a 10-min period, the donor islet cells were infused into a sigmoid or a branch of the left colic vein through a 22-gauge intravenous catheter, followed by a rinse with 50 ml of transplant medium. After completion of the infusion, the vessel was gently compressed for hemostasis. Before closure, the duodenum was inspected for adequate blood supply. The abdominal closure was done in one layer with interrupted sutures, using absorbable polyglactin 910 suture material. The skin was closed with interrupted vertical mattress sutures using nonabsorbable nylon suture material.

Postoperative care and diet. On the day of islet transplantation, baboons were given intravenous fluids. Buprenorphine hydrochioride (Buprenex, 0.05 mg/kg s.c.; Reckitt & Colman Pharmaceuticals, Richmond, VA) was administered for pain on the day of surgery and on postoperative day (POD) 1. Baytril antibiotic (enrofloxacin; Bayer, Agriculture Division, Animal Health, Shawnee Mission, KS) was given at a dose of 5 mg/kg i.m. on PODs 0-4. On POD 1, the animals were given Gatorade (Quaker Oats, Chicago). Water was administered ad libitum. Fruit was given on POD 2, and banana mixed with biscuit crumbs on POD 3. The animals were subsequently fed a morning and an afternoon meal of High Protein Monkey Chow (product code #5045; Purina Mills, Richmond, IN) and fruit (total daily intake: 2-4% body wt, including 47.9% carbohydrates, 5% fat, 25% protein, and 6% fibers). Pancreatic exocrine insufficiency, resulting from the pancreatectomy procedure that was used to create diabetes, was compensated with Viokase-V (Fort Dodge Animal Health, Fort Dodge, IA). Except for the animals that rejected islets early (in the first 2 weeks posttransplant), all baboons gained weight (up to 1 kg); none experienced steatorrhea.

Insulin. Diabetic baboons with no endogenous insulin secretory capacity required 4-6 U of insulin (multiplied by) (kg.sup.-1) (multiplied by) (day.sup.-1) to maintain blood glucose levels in the 100-200 mg/dl range and had an absence of stimulated C-peptide production. When necessary, baboons were treated with Humulin R (Eli Lilly, Indianapolis, IN) to maintain blood glucose levels in the 100-200 mg/dl range. In some cases, reduced dosages of exogenous insulin ((is less than) (multiplied by) (kg.sup.-1) (multiplied by) (day.sup.-1), compared with ~6 U (multiplied by) (kg.sup.-1) (multiplied by) (day.sup.-1) for diabetic baboons that were completely deficient in insulin/C-peptide secretion) were administered for 7-10 days posttransplant or for variable periods of time after a rejection episode to optimize the conditions necessary for successful engraftment or recovery from rejection, respectively. Subsequent to a rejection episode, insulin was gradually discontinued as follows. The total daily insulin dose was decreased, and the baboon was observed for stability of metabolic control for 1-3 days, followed by a further dose decrease and continued observation. This process was continued until the animal no longer required insulin to sustain euglycemia. Considering the absence of a pancreas, we then considered the islet allografts to be functionally competent, and the recipients were labeled "insulin independent." For animals that had experienced multiple or severe rejection episodes, the functional islet mass was no longer adequate to allow for insulin independence; many of these baboons, however, had clinically relevant graft function. Animals were considered to have partial graft function if fasting C-peptide was (is greater than) 0.5 ng/ml, response to intravenous glucose uncovered augmented insulin and C-peptide secretory responses, and reduced dosages of

exogenous insulin ((is less than) 4.0 U (multiplied by) (kg.sup.-1) (multiplied by) (day.sup.-1), compared with 4-(; U (multiplied by) (kg.sup.-1) (multiplied by) (day.sup.-1) for animals with no endogenous insulin secretory capacity) were required to maintain blood glucose levels between 100 and 200 mg/dl. The ease of maintaining normoglycemia was related to the functional islet mass: i.e., it was possible to control glycemia with very small doses of insulin in all but two animals (BA4)4 and -06).

Bone marrow preparation and administration. Vertebral bodies were harvested from the pancreas donor and processed to obtain donor bone marrow cells (DBMC) via modification of methods for processing of human vertebral bodies (45). DBMC were cryopreserved and were thawed immediately before intravenous infusion on PODs 5 and 11. A total of (10.sup.9) nucleated DBMC were administered per kilogram of recipient body weight.

Immunosuppression. Tacrolimus was administered at a dose of 0.1 mg (multiplied by) (kg.sup.-1) (multiplied by) (day.sup.-1) i.m., starting on POD -7. Drug levels were monitored daily, and dosage was adjusted to maintain trough levels of ~7-15 ng/ml. Animals were observed for signs of toxicity (neurotoxicity, hyperkalemia), and dosage was adjusted accordingly.

hu5c8 monotherapy. Humanized anti-CD154 (hu5c8) was obtained from Biogen. The production and characterization of the 5c8 clone has been described (38). Induction therapy consisted of intravenous administration of 10 or 20 mg/(kg.sup.-1) hu5c8 on PODs -1, 3, and 10. For treatment of rejection, 20 mg/(kg.sup.-1) hu5c8 was administered on the day rejection was detected, as well as 4 and 11 days thereafter. For BA4)5 and 4)6, 20 mg/kg maintenance therapy was given every 28 days for 6 months, beginning on POD 28. BA4)3 was switched to monthly maintenance therapy after POD 200. Blood samples were drawn periodically to assess levels of hu5c8 and anti-5c8 by enzyine-linked immunosorbent assay (Biogen).

Glucose monitoring and definition of rejection. In most cases, fasting and postprandial glucose (FG and PPG, respectively) were monitored via heel stick and blood testing with test strips and a Glucometer Elite (Bayer, Elkhart, IN). PPG was defined as the glucose reading obtained 2-3 h after removal of the morning meal. Baboons were not sedated with ketamine to obtain these readings. As a weekly routine, blood samples were collected to obtain plasma for testing of FG levels on a Beckman glucose analyzer (Beckman, Brea, CA). Plasma samples were also tested to confirm any high blood glucose readings. We initially defined islet failure (whether from primary nonfunction or rejection) as two consecutive days of fasting plasma glucose of (is greater than or equal to) 250 mg/dl (normal FG = 86 (+ or -) 4 (46)). During the course of these studies, however, it became evident that postprandial hyperglycemia preceded elevations in FG for animals with prolonged islet allograft function. After our experience in the first set of baboons, we modified our definition of islet failure to two consecutive FGs (is greater than) 100 and/or two consecutive PPGs (is greater than) 150 mg/dl.

Intravenous glucose tolerance test. Intravenous glucose tolerance tests (IVGTTs) were carried out after a 16- to 18-h overnight fast. The tests were performed as previously described (46). In brief, blood samples were collected at-10, -1, and 0 min, followed by injection (over a 20-s period) into the saphenous vein of 0.5 g glucose, in a 50% glucose solution, per kilogram of body weight. Samples of 1.5 ml were collected from the contralateral femoral artery at 1, 3, 5, 7, 10, 15, 20, 25, and 30 min postinjection, for a total of 12 blood samples over a 40-min period. Samples were drawn into glass tubes containing 0.5 ml 15% fluid EDTA and 0.2 ml trasylol (500 kallikrein inhibitor units aprotinin/ml blood), placed on ice, and centrifuged within 10 min. Plasma was then frozen at-80 (degrees)C and assayed later for glucose and immunoreactive insulin.

Additional monitoring. Before the first dose of anti-CD154 (hu5c8), and periodically posttransplant, blood samples were collected for complete blood counts and differential phenotypic analysis of leukocyte subsets by

flow cytometry, analysis of serum chemistries, and measurement of fasting plasma insulin, glucose, and C-peptide. For recipients of donor marrow, samples were also collected for assessment of chimerism . The collection of blood samples via venipuncture was accomplished with intramuscular injection of ketamine hydrochloride (Ketaset; Fort Dodge Laboratories) at a dose of 5 mg/kg for chemical restraint. Prolongation of sedation was achieved with additional doses of 5 mg/kg. Ketamine administration results in a reduction of insulin secretion in some, but not all, experimental animals (46); thus, ketamine supplementation, when required, was kept as low as possible.

RESULTS

Diabetes was induced in all recipients by total pancreatectomy (see METHODS). As shown in Fig. 1A, intrahepatic transplantation of allogeneic islets into a nonimmunosuppressed baboon, or into a baboon treated with tacrolimus, successfully but transiently restored normogglycemia and insulin independence. Within 8 days, however, both baboons acutely developed fasting hyperglycemia, reflecting allograft failure. In contrast, three of three baboons treated with 20 mg/kg humanized anti-CD154 (hu5c8) on PODs -1, 3, and 10 were insulin independent and normoglycemic for the first 30 days posttransplant (BA-01, -02, and -03; Fig. 1). In the absence of additional immune intervention, rejection episodes, first detected as elevations in PPG (followed by elevated FG on POD 59), occurred on POD 58 for two of three animals (BA-01 and -02) and on POD 31 for the other baboon (BA-03). Two additional baboons were treated with reduced doses of 10 mg/kg hu5c8 induction therapy (BA-04 and -05). One of these baboons (BA-04) did not experience rejection until POD 58, while the other baboon (BA-05) rejected the islets on POD 10 (Fig. 1A). There was no correlation between the day of rejection and the number of IEQ/kg.

(Figure 1 ILLUSTRATION OMITTED)

When hyperglycemia developed in BA-01, -02, -03, and -04, they were given anti-rejection therapy consisting of three additional doses of anti-CD154 (hu5c8) and small doses of exogenous regular insulin (Humulin R,(is less than) 1 U (multiplied by) (kg.sup.-1)(multiplied by)(day.sup.-1)) for several days to stabilize metabolic control during recovery. Insulin was gradually tapered as follows: the dose of insulin (per day) was reduced, the animal was observed for stable metabolic control, and the dose was reduced again. The process was continued until the baboon no longer required insulin to sustain euglycemia, and the recipients were considered to be insulin independent. For comparison, pancreatectomized baboons that do not have functioning islet allografts require insulin doses of 4-6 U (multiplied by) (kg.sup.-1) (multiplied by) (day.sup.-1) to maintain blood glucose values in the 100-200 mg/dl range.

Anti-CD154 appeared to effectively halt rejection in all four baboons. One of the animals (BA-01; Fig. 1) was euthanatized on POD 79 to obtain tissues for immunohistological analysis. Several intrahepatic islets with intact insulin-positive cells were detected (Fig. 2A). Some islets were surrounded by noninfiltrating (CD4.sup.+) (Fig. 2B) and (CD8.sup.+) lymphocytes, and some areas of apparent islet loss were also evident (data not shown). Before the baboon was euthanatized, and to test whether euglycemia resulted from functioning intrahepatic, islets, an IVGTT was performed on BA-01 with simultaneous sampling from both the portal vein and the suprahepatic vena cava. As shown in Fig. 3, we observed a reversal of the physiological portal to a systemic gradient of both basal and stimulated insulin: i.e., higher insulin concentrations in the suprahepatic vena cava, compared with the portal vein. These data confirm that the endogenous insulin secretion originated from intrahepatic islets. The other baboon in this pair was maintained on small doses of exogenous insulin ((is less than) 1 U. (kg.sup.-1) (multiplied by) (day.sup.-1) until POD 100 and was subsequently returned to insulin independence (BA-02; Fig. 1). This animal experienced additional rejection episodes, first detected by elevations in PPG, on PODs 118, 147, 179, 230, and 267, all of which were reversible with anti-CD154. After the last two rejection episodes, the

baboon had lost sufficient functional islet mass that continued small doses of exogenous insulin were required to maintain euglycemia (note the elevated FG levels subsequent to POD 179; Fig. 1B). The animal was euthanatized with a partially functioning graft, as reflected by the continued presence of fasting C-peptide ((is greater than) 0.5 ng/ml) and insulin-positive intrahepatic islets (data not shown), on POD 303. No residual pancreas was observed at autopsy.

(Figures 2-3 ILLUSTRATION OMITTED)

Subsequent to the resolution of rejection on POD 31, BA4)3 experienced additional reversible rejection episodes on PODs 112 and 172 (Fig. 1B), regained insulin independence, and was subsequently placed on monthly maintenance doses of 20 mg/(kg.sup.-1) anti-CD154 (hu5c8, given on PODs 200, 228, and 256). The baboon remained insulin independent, but experienced a rejection episode on POD 284 that appeared to be successfully reversed with anti-CD154 but resulted in a continued requirement for low-dose insulin to maintain normoglycemia. The animal was euthanatized on POD 301. As shown in Fig. 4, postmortem histological analysis of the intrahepatic islets revealed well-preserved insulin-positive cells, with rare inflammatory leukocytes.

(Figure 4 ILLUSTRATION OMITTED)

The remaining baboon (BA-04) experienced a rejection episode on POD 58, was retreated with anti-CD154 (hu5c8) and transient low-dose insulin, and did not experience an additional rejection episode until POD 264. In this case, rejection was rapid, acute, and not reversible (Fig. 5). At the time of rejection, and unlike the other three baboons, this recipient had not received any anti-CD154 therapy for 206 days. During the course of these studies, we observed that elevated PPG preceded the onset of fasting hyperglycemia; this is clearly reflected in Fig. 5. The PPG allowed us to detect acute rejection episodes on PODs 58 and 264, while the FG remained normal.

(Figure 5 ILLUSTRATION OMITTED)

IVGTTs were performed on BA-03 and BA-04, both pretransplant and at intervals posttransplant. The results revealed that glucose response curves were very similar at all time points in animals' with a functioning islet allograft. The first-phase insulin release (FPIR), however, decreased over time for both animals, strongly suggesting a loss of functional islet mass with each rejection episode (47). Representative results for glucose and insulin release after intravenous glucose challenge of BA-03 are given in Fig. 6. FPIR on POD 35 was lower than that observed 12 days before pancreatectomy and islet cell transplant and may reflect incomplete engraftment (e.g., due to incomplete revascularization) and a loss of functional islet mass subsequent to the rejection episode detected on POD 31. The results obtained at 149 days posttransplant revealed a decrease in FPIR (rejection episode detected at POD 112). At PODs 168 and 279, further reductions in FPIR were observed, coinciding temporally with clinically relevant rejection episodes on PODs 172 and 284, respectively.

(Figure 6 ILLUSTRATION OMITTED)

Subsequent to these initial experiments and the observation of a loss of functional islet mass temporally related to each episode of rejection, two baboons (BA-06 and -07) were treated with 20 mg/kg anti-CD154 (hu5c8) induction therapy on PODs -1, 3, 10, and 28, followed by monthly anti-CD154 (hu5c8) maintenance therapy at a dose of 20 mg/kg, given every 28 days. In addition to 5c8 therapy, these baboons received infusions of whole donor vertebral body marrow on PODs 5 and 11 (total dose of 109 nucleated cells/kg). These animals remained normoglycemic and insulin independent for 114 and 238 days.

Results of sequential IVGTT revealed that in the absence of rejection, FPIR was maintained in BA-06 and -07. Representative results are given in Fig. 7 for BA4)6. The pretransplant and POD 66 insulin response curves were very similar (Fig. 7), reflecting maintenance of functional islet mass in the absence of rejection. Performance of random IVGTT on POD 113 revealed a slight decrease in insulin release. Immediately thereafter,

we determined that the baboon was undergoing graft rejection. Our definition of graft rejection was FG (is greater than) 100 mg/dl and PPG (is greater than) 150 mg/dl. The PPG was (is greater than) 150 mg/dl on POD 114, and the FG increased to (is greater than) 100 mg/dl on POD 116. In this case, rejection was not reversible with additional anti-CD154 (hu5c8) therapy: i.e., the baboon was initially treated with reduced dosages of exogenous insulin ((is less than)1 U (multiplied by) (kg.sup.-1) (multiplied by) (day.sup.-1)), but the glucose levels and insulin requirement continued to increase (to (is (is greater than)6 U (multiplied by) (kg.sup.-1) (multiplied by) (day.sup.-1)) in association with progression of rejection. Insulin secretion in response to IVGTT on POD 154 (Fig. 7A) was undetectable, thus continuing that the intrahepatic islets had been rejected. In contrast, BA-07 experienced excellent metabolic control for over 6 months, and no rejection was detected via monitoring of FG and PPG. After the seventh maintenance dose on POD 198, the baboon was no longer given anti-CD154 (hu5c8) and subsequently experienced rejection on POD 239. The animal was euthanatized with clinically relevant graft function on POD 253.

(Figure 7 ILLUSTRATION OMITTED)

The overall results for duration of insulin independence for all transplanted baboons are summarized in Table 1.

TABLE 1 Prolongation of baboon islet allograft survival with anti-CD154

Group	n
Control	1
Tacrolimus	1
Anti-CD154 induction/anti-rejection	5
Anti-CD154 induction/maintenance + donor marrow	2

Group	Duration of insulin independence (day of death)
Control	8 (14)
Tacrolimus	8 (21)
Anti-CD154 induction/anti-rejection	10 (13)(*), 59 (79), 229 (303), 264 (300)(*), 284 (301)
Anti-CD154 induction/maintenance + donor marrow	114 (162), 238 (253)

Animals were euthanatized with varying degrees of graft function. (*) 10 mg/kg anti-CD154; all other baboons received 20 mg/kg therapy.

Two nontransplanted control baboons were treated with hu5c8 induction therapy. Before, and periodically after, treatment with anti-CD154, the animals underwent IVGTT. The administration of hu5c8 did not influence the results of IVGTT (data not shown).

While we did not observe any physical or behavioral evidence of drug-induced toxicity, blood samples were obtained pretransplant, and at various times posttransplant, for complete blood counts, flow cytometric analysis of peripheral blood leukocytes, and chemistries. All values were normal except for a marked decrease in the circulating (CD4.sup.+) lymphocyte cell numbers in all animals treated with anti-CD 154 (hu5c8). The percentage of (CD4.sup.+) T-cells ranged from 2 to 7% (whole blood lysis method, analysis of total leukocyte gate) in the 5c8 treated baboons, compared with a mean value of 11% in untreated animals. Similarly, in animals given anti-CD154 (hu5c8), 16-18% of lymph node cells were CD2/CD4 dual positive, compared with 44% in control animals. The decrease in (CD4.sup.+) cell numbers occurred -3 weeks after islet cell transplantation. Since total lymphocyte counts did not decrease, an increase in the percentage of (CD8.sup.+) T-cells was observed that was coincident with the decline in (CD4.sup.+) cell counts. We observed the same phenomenon in two normal nontransplanted baboons treated with

anti-CD154 (hu5c8) alone; after discontinuation of hu5c8 treatment, the (CD4.sup.+) T-cell counts gradually recovered. Interestingly, we detected CD8/CD69 dual positive cells in the peripheral blood of transplanted animals at ~2 weeks posttransplant. We were unable to measure any CD154 on peripheral blood T-cells harvested from these baboons at any time point, although in vitro activation with phorbol myristate acetate and ionomycin led to rapid (6-h) and transient (gone by 24 h) upregulation of CD154 on baboon (CD4.sup.+) T-cells (data not shown). As mentioned previously, (CD4.sup.+) lymphocytes were detected in biopsies taken from an animal that had recently undergone a rejection episode (BA-01), and this baboon had the lowest CD4 counts of all the animals tested. No adverse side effects, such as increased incidence of infection, were observed in association with lowered peripheral CD4 counts. In addition, no alteration in leukocyte subsets has been detected in rhesus monkey recipients of allogeneic kidneys (13) or islets (N.S.K., M.C., M.M., A.R., M. Oliveira, J.L.W., A.D.K., D.M.H., L.C.B., C.R., unpublished observations) or in cynomolgous monkeys treated with anti-CD154 (hu5cS) (Biogen).

All recipients were MLC reactive against their donors pretransplant. For two animals tested within the first month posttransplant, anti-donor-specific MLC reactivity was maintained (BA-01 and BA-02). As shown in Table 2, periodic MLC screening at various intervals posttransplant revealed decreased reactivity to donor and third-party cells, as well as decreased proliferative responses to phytohemagglutinin, for all recipients after the first month posttransplant. Anti-donor-specific MLC reactivity was decreased to background levels for all but one animal (BA-06), which experienced irreversible rejection within 12 days of the MLC test. Anti-third-party reactivity was maintained above background, as was proliferative responsiveness to PHA (Table 2).

TABLE 2 Proliferative responses to donor and third-party cells and PHA

Animal	Anti-self	Anti-donor	Anti-third	PHA
BA-01				
Pretransplant	3,404	42,262	41,091	97,504
POD 23	2,565	29,008	ND	ND
BA-02				
Pretransplant	3,149	43,545	46,424	96,308
POD 23	3,374	37,456	ND	62,897
POD 198	1,258	1,439	8,217	13,757
BA-03				
Pretransplant	2,715	38,653	43,224	68,619
POD 119	162	1,785	3,464	3,360
BA-04				
Pretransplant	2,605	39,687	41,357	110,431
POD 90	372	1,700	2,008	ND
POD 119	423	1,614	5,743	12,190
BA-06				
Pretransplant	1,033	35,235	46,783	37,616
POD 72	4,384	4,048	6,755	8,674
POD 101	6,204	14,350	8,915	12,190
BA-07				
Pretransplant	1,247	25,579	30,773	124,399
POD 72	3,500	9,832	5,883	ND
POD 101	775	1,505	5,125	12,190

Data are counts per minute of incorporated ((sup.3) H)thymidine. ND, not determined.

Development of antibodies specific for humanized anti-CD154 (hu5c8) monoclonal antibody was not detected in any baboon during the treatment period; however, for animals that remained off therapy for several months (two antibody-treated nontransplanted control animals and BA-04), low titers of anti-5c8 were detected (data not shown).

DISCUSSION

Costimulatory blockade, with agents that block the B7:CD28 and/or the CD40:CD154 pathways, has proven to be an effective means to promote allograft acceptance in various rodent models, and has more recently been shown to promote acceptance and long-term survival of renal allografts in rhesus monkeys (13). Blockade of the B7:CD28 pathway via treatment with CTLA4-Ig has been shown to prolong the survival of allogeneic islets, compared with control animals, in a cynomolgous monkey model (48). In murine models, islet allograft acceptance via therapy with anti-CD154 has required infusion of donor-derived hematopoietic cells (21,22).

Our data demonstrate a uniform effect of CD40:CD154 costimulatory blockade on engraftment and long-term survival (up to 300 days) of allogeneic islets in a baboon model. In contrast to the murine data, infusion of donor-derived cells was not necessary. In fact, infusion of donor vertebral body marrow on PODs 5 and 11 did not add to the graft-promoting effect of anti-CD154 (hu5c8) alone. Alteration of the timing of marrow infusion may, however, yield different results and is currently under study.

The observation that elevations in PPG preceded increases in FG and were correlated with rejection enabled us to detect and reverse islet allograft rejection in most cases. The results of this study demonstrate that intrahepatic islet allografts can successfully engraft and survive long term and that preventing rejection allows for preservation of functional islet mass. We can think of several possible explanations for our success, and all may contribute. First, in the presence of costimulatory receptor blockade with anti-CD154, rejection is prevented in this model. Several groups have recently suggested that T-cell help is delivered to (CD8.sup.+) cells via dendritic cells and that (CD40.sup.+) dendritic cells are activated by (CD154.sup.+) CD4 cells (49-51). Thus, anti-CD154 could function, at least in part, by impairing the functional maturation of (CD8.sup.+) T-cells. The detection of CD8/CD69-positive T-cells in the initial posttransplant period suggests that early activation events had occurred in the CD8 subset, yet islets were not rejected, consistent with the notion that CD8 functional maturation is impaired.

A second possible reason-for these findings is the reported ability of anti-CD154 to prevent macrophage- and endothelial cell-mediated production of nitric oxide and proinflammatory cytokines (14-18). Costimulatory blockade with anti-CD154 (hu5c8) may, therefore, play a key role in preventing the early islet loss thought to occur subsequent to intrahepatic transplantation (52,53). To capitalize on this possibility, islets were transplanted in medium containing 20 mg of antiCD154 (hu5c8).

Third, our in vivo studies have demonstrated that hu5c8 does not have any direct adverse effect on islet cell function. Fourth, the deletion of CD4+ cells we observed could have contributed to islet engraftment and survival. While this possibility cannot be completely refuted, we observed rejection episodes in baboons with low peripheral CD4 cell counts, and these episodes, when detected early, were reversed with anti-CD154 (hu5c8) monoclonal antibody readministration and did not result in a further decrease in (CD4.sup.+) cell counts. Furthermore, (CD4.sup.+) cells were detected in the infiltrate surrounding intrahepatic islets subsequent to a rejection episode.

In summary, costimulatory blockade with anti-CD154 monotherapy allows for islet engraftment, insulin independence, and long-term function in baboon recipients of allogeneic islets. Reversal of rejection can be achieved with repeated administration of anti-CD154, and prevention of rejection allows for preservation of functional islet mass and insulin independence. These observations on costimulatory blockade in nonhuman primates with islet transplants are sufficiently unique to stimulate further study of this and other approaches, alone or in combination, to intervention in the activation signals responsible for allograft rejection.

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APC, antigen presenting cells; DBMC, donor bone marrow cells; FG, fasting glucose; FPIR, first-phase insulin release; IEQ, islet equivalent; IVGTT, intravenous glucose tolerance test; MLC, mixed leukocyte culture; PBMC, peripheral blood mononuclear cells; POD, postoperative day; PPG, postprandial glucose.

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Immunosuppressive strategies in transplantation.
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TEXT:

In the absence of immunosuppression, transplanted organs invariably undergo progressive immune-mediated injury. Over the past 40 years, immunosuppressive drug regimens have evolved greatly and transformed solid-organ transplantation into a routine clinical procedure with impressive short-term results obtained in kidney, heart, lung, liver, and pancreas transplantation. 1-year renal allograft (transplant) survival in most centres is now between 80% and 90%; short-term graft survival rates for other organs are approaching this proportion. Improved short-term survival is a consequence mainly of better prevention and treatment of acute rejection.

By contrast, long-term graft survival remains a major problem. Late graft loss is caused predominantly by chronic rejection -- a poorly defined clinicopathological event that is immunologically mediated but unresponsive as yet to immunosuppressive therapy. Moreover, the side- effects (such as nephrotoxic effects, hypertension, and hyperlipidaemia) of some immunosuppressive agents have been implicated in the pathogenesis of chronic allograft loss. Current immunosuppressive agents also lack

specificity; any reduction in immune responsiveness to the allograft is accompanied by reduced immunity to infection and malignant disease. The search continues, therefore, for the ideal immunosuppressive agent -- one that selectively inhibits alloantigen immune responses, prevents chronic allograft rejection, and is free of major side-effects.

With the recent explosion in knowledge of molecular immunology, major advances have been made in understanding of the cellular and molecular mechanisms that underlie the immunological response to transplanted organs. These advances have enabled a more comprehensive understanding of how established immunosuppressive drugs work, and have provided insight into how better agents might be developed. The purpose of this review is to provide a basic understanding of the immunological response to a transplanted organ, to outline how established and recently approved immunosuppressive agents work, and to discuss the direction of future immunosuppressive strategies.

Mechanisms of allograft rejection: central role for T cells

The CD4 T cell is crucial in both the initiation and the coordination of the rejection response (figure 1). The recipient mounts a rejection response following CD4 T cell recognition of foreign antigens derived from the allograft.1,2 These antigens are encoded predominantly by highly polymorphic loci within the major histocompatibility complex on the short arm of chromosome 6.3 The structural basis of T cell receptor binding to peptide in the major histocompatibility complex groove has been solved.4 In the setting of transplantation, recipient T cells may recognise intact foreign major histocompatibility complex encoded molecules on donor cells (direct allorecognition) or peptides derived from foreign major histocompatibility complex molecules, shed from the graft and subsequently processed and presented, bound to self major histocompatibility molecules, by recipient antigen-presenting cells (indirect allorecognition).5 Direct allorecognition can activate a much larger proportion of the T cell pool and may cause the vigorous immune response in acute rejection.6 Indirect allorecognition generates smaller numbers of alloreactive T cell clones, and several lines of evidence suggest that this pathway may lead to the insidious immune response that occurs in chronic rejection.7

Antigen recognition alone is insufficient to activate fully the CD4 T cell; a second (costimulatory) signal must be provided by cognate ligands on the antigen-presenting cell. When both signals are provided, the T cell secretes optimum concentrations of interleukin 2 -- a potent autocrine growth factor that induces T cell proliferation, clonal expansion, and cytokine production. In the absence of the second signal, the CD4 T cell becomes unresponsive to further exposure to antigen, fails to secrete cytokines, and may undergo programmed cell death (apoptosis).8,9 This dependence on a second signal forms the basis for use of agents to block CD4 T cell costimulation to induce allograft tolerance in vivo.2 The best-characterised costimulatory signal is provided by ligation of CD28 on the surface of the CD4 T cell with a member of the B7 family of molecules (B7-1 or B7-2) on the antigen-presenting cell.2,10 CD40-CD154 interactions provide another important costimulatory signal for T cell activation.11,12 A mutational deficiency of CD154 (CD40 ligand) is the cause of the hyper-IgM syndrome in which T cell defence against intracellular microorganisms is impaired.13

Alloactivated CD4 T cells subsequently interact with effector cells of the rejection response via direct cell-cell contact and cytokine secretion. By an increase in the activation and function of B cells, cytotoxic CD8 T cells and monocyte/macrophages, alloactivated CD4 T cells promote alloantibody production, antigen-specific cell lysis, and delayed type hypersensitivity responses, respectively. These effector mechanisms ultimately result in graft destruction (figure 1).

New and established immunosuppressive agents

All current immunosuppressive drugs target T cell activation and cytokine production, clonal expansion, or both (figure 2).14 Standard transplantation immunosuppressive protocols in current use consist of

initial and maintenance therapies to prevent allograft rejection and short courses of more aggressive immunosuppressive therapy to treat episodes of acute rejection. Treatment of acute rejection is not reviewed further here.

Initial immunosuppression

Immunosuppression is initiated at high levels in the immediate post-transplant period when the risk of graft rejection is greatest. In most patients, initial immunosuppression consists simply of higher doses of the agents used in maintenance therapy. Induction immunosuppression, however, involves the additional administration of potent anti-T-cell antibody preparations (table 1). This procedure is considered mainly for individuals at higher risk of acute rejection, such as children or sensitised patients (eg, those having second or subsequent transplants, those with previous pregnancies, or those who have received multiple blood transfusions). Also, in the setting of post-transplant acute tubular necrosis, induction therapy allows for delayed introduction of full-dose cyclosporin or tacrolimus. In this setting, the nephrotoxic effects of high-dose cyclosporin or tacrolimus may extend the duration of acute tubular necrosis.

Standard induction therapy consists of a 7-14 day course of either polyclonal antilymphocyte antibodies (antithymocyte globulin or antilymphocyte globulin) or monoclonal anti-CD3 antibodies. These agents are successful in reducing the incidence and severity of early acute rejection.15 However, routine use of these antibody preparations is not indicated because their potent immunosuppressive effect may significantly increase the risk of serious infection or malignant disease.15,16 These complications are most commonly observed with repeated administration.16 Specific side-effects and the mechanisms of action of these agents are listed in table 1. With the introduction of more potent orally active maintenance immunosuppressive agents, the need for induction therapy with polyclonal antilymphocyte antibodies or monoclonal anti-CD3 antibodies is diminishing.

Trials have confirmed the effectiveness of monoclonal antibodies directed against the interleukin-2 receptor as a component of initial immunosuppressive therapy. Interleukin-2-receptor blockade may provide more specific immunosuppression since the interleukin-2 receptor is expressed only by activated lymphocytes. In initial clinical studies, rodent antibodies to interleukin-2 receptor administered as induction therapy were as effective as, and better tolerated than, antithymocyte globulin,17 and allowed safe use of low initial doses of cyclosporin.18 The short plasma half-life of these antibodies and the generation of human anti-rodent antibodies by the recipient limited the efficacy of these preparations to several days. These difficulties have been overcome through the development of chimeric and humanised antibodies to interleukin-2 receptor, in which only portions of the antibody binding site are derived from the original rodent antibody, and the remaining portions are human in origin. Two antibody preparations, basiliximab and daclizumab, have undergone phase III clinical studies.19,20 Both preparations were compared with placebo in combination with standard cyclosporin and corticosteroids (and with azathioprine in the dacluzimab trial). The results of these trials were strikingly similar: both antibodies were well tolerated and reduced the incidence of acute rejection by roughly 35%; neither increased the incidence of infection or other adverse events within the 1-year follow-up period. Although these agents are given only for a limited period after transplantation, effective interleukin-2-receptor blockade is achieved for several weeks after the last dose, thereby covering the critical period when acute rejection is most common. Several questions remain unanswered. First, will these agents still show a benefit when combined with more potent immunosuppressive agents such as mycophenolate mofetil and tacrolimus? Second, will interleukin-2-receptor blockade circumvent the need for calcineurin inhibitors (cyclosporin or tacrolimus and their side-effects), at least in a subset of patients, since both classes of drugs ultimately block T cell activation induced by interleukin 2? Preliminary data, however, show a fairly high rate of acute rejection in

patients receiving anti-interleukin-2 receptor antibodies, mycophenolate mofetil, and steroids in the absence of calcineurin inhibition.21

Maintenance immunosuppression

Maintenance immunosuppression is best achieved with combinations of immunosuppressive agents. Combination therapy aims to minimise the side-effects of any single drug whilst maintaining adequate overall immunosuppression by targeting multiple steps in T cell activation (figure 2). This is usually achieved by combining corticosteroids with a calcineurin inhibitor (cyclosporin or tacrolimus) and an antiproliferative agent such as azathioprine or mycophenolate mofetil (table 2). At present, the overall level of maintenance immunosuppression is determined mainly by the perceived risk of rejection. Factors such as type of organ transplanted, degree of HLA incompatibility, recipient race, and history of previous acute rejection all influence the choice and dosage of specific maintenance immunosuppressive agents. Hopefully, an improved understanding of the immunobiology of allograft rejection and the development of molecular techniques to monitor the host antigraft response may allow for more accurate titration of immunosuppression.22 For example, Hutchinson and colleagues23 have identified certain cytokine gene polymorphisms in transplant patients that are associated with altered cytokine production and with increased risk of allograft rejection.24 The limitations of our current ability to balance the risks of over- immunosuppression and under-immunosuppression are highlighted by the fact that 30-40% of transplant recipients are predicted to develop neoplasia over 30 years.25 Evolving maintenance immunosuppressive regimens are listed in table 3.

Calcineurin inhibitors

Calcineurin inhibitors are currently the keystone of most immunosuppressive regimens used in clinical organ transplantation. Both cyclosporin and tacrolimus bind to cytoplasmic receptors (cyclophylin and FK-binding protein 12 (FKBP-12), respectively) and resulting complexes inactivate calcineurin, a pivotal enzyme in T cell receptor signalling. Calcinerin inhibition prevents interleukin-2 gene transcription, thereby inhibiting T cell interleukin-2 production26 (figure 2). The introduction of cyclosporin proved an important advance in immunosuppressive therapy and increased 1-year graft survival for cadaveric kidney allografts by 10%.27 However, therapy with cyclosporin is associated with several important side-effects owing in part to the ubiquitous tissue distribution of cyclophylins (table 2). Such side-effects include acute and chronic nephrotoxic effects, hypertension, and dyslipidaemia. Acute cyclosporin nephrotoxic effects occur secondary to intrarenal vasoconstriction and may exacerbate acute tubular necrosis in cadaveric renal transplantation. Acute nephrotoxic effects from cyclosporin are reversible; chronic effects are not. Chronic cyclosporin nephrotoxic effects are probably a long-term sequela of persistent renal vasoconstriction and ischaemia, but induction of the potent fibrogenic growth factor, transforming growth factor-b, may also contribute.28,29 Such toxic effects are characterised histologically by obliterative vasculopathy and interstitial fibrosis.

To minimise the toxic effects of cyclosporin yet maintain adequate immunosuppression, cyclosporin dosage must be tailored to each patient. Monitoring of trough concentrations is essential. Although high doses of cyclosporin are advocated in the initial 6-12 months after transplantation, the appropriate long-term dosage is contested.30 Registry data suggest that lower maintenance doses are associated with poorer graft outcome.31 However, a recent prospective trial32 has shown that lower target cyclosporin concentrations did not compromise long-term (5 year) graft survival but were associated with a reduction in the incidence of neoplasia.32 A major difficulty with the standard oil-based formulation of cyclosporin is poor and variable oral absorption resulting in large variability between patients in total cyclosporin exposure: this is a factor that may contribute to poorer graft outcome in some patients.33 This difficulty led to the development of a new microemulsion formation of cyclosporin, called Neoral. This formulation has both better and more

consistent bioavailability, which results in more reproducible daily exposure to cyclosporin. There is evidence that this improved pharmacokinetic profile translates into lower acute rejection rates.34 Initial worries that increased bioavailability might lead to an increase in long-term toxic effects have not been borne out in large multicentre studies.35 Whether patients stabilised on standard cyclosporin should be converted to the microemulsion formulation depends mainly on patient's and physician's preference. The introduction of generic formulations of cyclosporin will probably compound this issue of choice.

Tacrolimus (FK506) has been developed as an alternative agent to cyclosporin,36 but its exact role as primary maintenance therapy outside the area of liver transplantation is not yet clearly defined. Tacrolimus is more potent than cyclosporin, presumably owing to a greater affinity of its complex with FKBP-12 to calcineurin.36 Tacrolimus is widely used in liver transplantation, in which its greater water solubility and lesser dependence on bile salt absorption results in improved bioavailability over cyclosporin (standard formulation). Large US and European multicentre trials in both liver and kidney transplantation show that tacrolimus-based immunosuppression is associated with a lower incidence of acute rejection than standard cyclosporin-based therapy.37-39 In addition, 3-year follow-up analysis of the US renal transplant study shows improved graft survival in tacrolimus-treated patients.40 Tacrolimus is also yielding impressive preliminary results when used in combined kidney and pancreas transplantation, and compares favourably with cyclosporin in the limited heart and lung transplantation studies to date. In renal transplantation, tacrolimus is commonly used as so-called rescue therapy for the treatment of refractory acute rejection in patients on cyclosporin-based therapy. Studies report a 75% rescue rate with tacrolimus with a mean 5-year follow-up period.41 This capacity to reverse refractory acute rejection is not evident with cyclosporin. Finally, tacrolimus and cyclosporin have different side-effect profiles: although they are of roughly equivalent nephrotoxicity, tacrolimus-treated patients have a higher incidence of diabetes mellitus and neurotoxic reaction, but a lower incidence of hypertension, hyperlipidaemia, hirsutism, and gum enlargement.38 Familarity with this new drug in transplant centres will undoubtedly reduce the risk of complications associated with its use. Continuing trials are comparing tacrolimus with Neoral as maintenance therapy in liver and kidney transplantation.

Antiproliferative agents

Antiproliferative agents prevent the expansion of alloactivated T cell and B cell clones. Azathioprine, a purine analogue that inhibits DNA synthesis, has been used as an immunosuppressive agent since the 1960s. Its principal side-effect is bone-marrow suppression. In many centres, mycophenolate mofetil is now replacing azathioprine in standard immunosuppression protocols for new kidney and pancreas-kidney transplants.42 The rationale for this switch is that mycophenolate mofetil is a selective inhibitor of the de-novo pathway of purine biosynthesis, thereby providing more specific and potent inhibition of T cell and B cell proliferation. Three large multicentre trials have compared mycophenolate mofetil with azathioprine or placebo in renal transplant patients receiving cyclosporin and corticosteroids.43 These studies show that mycophenolate mofetil therapy is associated with a lower incidence of acute rejection (50% reduction) in the first year after transplantation, and a lower requirement for high-dose steroids and antilymphocyte antibody therapy to treat rejection.44 However, comparative studies so far (over 3 years follow-up) have not shown improved long-term graft survival with mycophenolate mofetil, perhaps owing to lack of adequate statistical power.45 In cardiac transplantation, a prospective randomised trial has shown a benefit of mycophenolate mofetil over azathioprine when results were examined on a treated but not on an intention-to-treat basis.46 Provisional renal-transplantation data suggest that mycophenolate mofetil may be useful in the treatment of acute cellular rejection,47 and in

reversing refractory acute rejection in patients on azathioprine.48 In animal models, mycophenolate mofetil prevents the development of chronic rejection;49 early observations suggest that it may have a similar effect in human transplantation.50 Side-effects of mycophenolate mofetil include gastrointestinal upset and diarrhoea (which may be dose-limiting) and an increased risk of tissue invasive cytomegalovirus infection. Interestingly, studies have not shown a reduced incidence of bone-marrow suppression with mycophenolate mofetil compared with azathioprine.

Sirolimus (rapamycin) is a highly potent immunosuppressive agent currently undergoing phase III clinical trials.51 Whereas cyclosporin and tacrolimus inhibit T cell production of cytokines, sirolimus inhibits T cell responses to these cytokines. Sirolimus inhibits intracellular signalling distal to the interleukin-2 receptor and inhibits progession of the T cell into the S phase of the cell cycle.51 This drug is designed for use with cyclosporin rather than with tacrolimus because both sirolimus and tacrolimus bind to the same intracellular protein (FKBP-12), although an antagonist effect in vivo is disputed.52 Phase I trials showed that sirolimus is well tolerated, with hyperlipidaemia and thrombocytopenia being the main side-effects.53 A phase II trial has shown that addition of sirolimus to a cyclosporin and corticosteroid regimen is associated with a less than 10% incidence of acute renal allograft rejection, and 1-year follow-up data suggest that steroid withdrawal can be achieved successfully in the majority of patients on this regimen.54 Such low rates of acute rejection can be maintained even when low doses of cyclosporin are used. Finally, a phase III European multicentre trial in renal transplantation is currently comparing sirolimus with cyclosporin used with azathioprine and corticosteroids. Preliminary data show similar incidences of acute rejection between groups, albeit higher than when both agents are combined.55 Experimental data suggest that sirolimus may prevent the development of graft atherosclerosis, the sine qua non of chronic rejection.56 This encouraging finding may be related to a non- specific antiproliferative effect of the drug.

Corticosteroids

Corticosteroids are non-specific anti-inflammatory agents. They inhibit cytokine production by T cells and macrophages, thereby disrupting T cell activation and macrophage-mediated tissue injury. This effect is mediated through inhibition of nuclear factor k B activation57 and by binding to glucocorticoid response elements in the promoter regions of cytokine genes. Corticosteroids have numerous well-known side-effects (table 2). Hypertension, dyslipidaemia, and glucose intolerance are side-effects shared by both corticosteroids and calcineurin inhibitors and undoubtedly contribute to the high prevalence of cardiovascular disease in transplant recipients. The fact that cardiovascular disease is the major cause of death in this population of patients must be stressed.58 These complications have prompted investigators to assess the effects of gradual corticosteroid withdrawal in stable renal allograft recipients maintained on cyclosporin and azathioprine. Most studies show that corticosteroid withdrawal 1 year after transplantation is associated with a small but significant risk of acute rejection.59 Furthermore, the potential deleterious effects of steroid withdrawal on long-term graft function have raised concerns over this practice.60 Hopefully, the development of immunological monitoring techniques may allow the identification of patients in whom safe withdrawal of steroids is achievable. In addition, introduction of newer agents such as tacrolimus, mycophenolate mofetil,61 and sirolimus54 may allow safer withdrawal of steroids (table 4), an issue currently being studied.

New immunosuppressive strategies in transplantation

Given the central role of the CD4 T cell in allograft rejection, it is not surprising that most new immunosuppressive strategies have sought to inhibit CD4 T cell activation. The ultimate aim would be to inhibit only those T cells that respond to donor antigen, thus achieving immunological unresponsiveness to the transplant in the face of a fully functional immune

system (donor-specific tolerance). Several ligand/receptor interactions occur between the T cell and the antigen-presenting cell during antigen presentation (figure 3). While some simply mediate cell- cell adhesion, others transduce activational signals to either the T cell or the antigen-presenting cell. Agents have been developed to block these interactions (table 3).

Blockade of T cell costimulation

An area of extensive research is the role of T cell costimulatory blockade in preventing transplant rejection.2 A critical property of costimulatory signals is the prevention of T cell anergy.8 Thus, blocking T cell costimulation soon after transplantation may render the recipient's CD4 T cells unresponsive to donor antigen. CD28/B7 interactions can be blocked effectively by CTLA4Ig. This molecule is formed by fusing the extracellular binding domain of soluble CTLA4 (a molecule encoded by a gene that is highly homologous to CD28) to the constant region of a human IgG1. CTLA4Ig binds B7-1 and B7-2 with greater affinity than CD28 and therefore acts as a potent competitive inhibitor of CD28-mediated T cell activation.62

Almost all studies of the effects of CD28-B7 blockade in animal models of transplantation have shown that a short course after transplantation extends allograft survival. In some models, the development of indefinite graft survival or tolerance with CD28-B7 blockade requires concomitant administration of donor antigen such as donor splenocytes63 or bone marrow.64 A corollary of the tolerogenic effects of CD28-B7 blockade is that CTLA4Ig can prevent65 and possibly interrupt66 the development of chronic rejection in experimental models. Interestingly, in one model, cyclosporin abrogates the effect of CTLA4Ig to prevent chronic rejection.67

Much research has focused on the effects of interrupting the CD40-CD154 costimulatory pathway in models of transplantation.2,12 CD40/CD154 interactions are critical in the activation of both CD4 T cells and B cells. Furthermore, signalling through CD40 has proinflammatory effects on endothelial cells and macrophages, suggesting that blockade of CD40/CD154 interactions may be beneficial in the prevention of chronic rejection.12 Combined blockade of the CD28/B7 and CD40/CD154 pathways is particularly effective, as was shown in a fully allogeneic mouse model of skin and cardiac transplantation.68

Primate studies suggest that costimulatory molecule blockade is a feasible strategy in human transplantation . Long-term graft survival has been obtained in non-human primate models of pancreatic cell69 and renal transplantation .70 In the latter study, the combination of CTLA4Ig and anti-CD154 (anti-CD40 ligand) was more effective than either agent alone. Furthermore, phase I trials in psoriasis, a T-cell-mediated disorder, provide evidence that CTLA4Ig is well tolerated and can inhibit T cell responses in human beings. Anti-CD154 monoclonal antibodies have been administered to patients with idiopathic thrombocytopenia, and studies in patients with systemic lupus erythematosus are planned. Timing of administration, duration of treatment, and use of concurrent immunosuppressive therapy are issues that need to be resolved before these agents are used in clinical organ transplantation .

Blockade of T cell adhesion molecules

Adhesion molecules are critical in the activation and recruitment of immune cells into the allograft. Antibodies directed against intracellular adhesion molecule-1 inhibit CD4 T cell activation in vitro and extend allograft survival in animal models. The increased expression of adhesion molecules such as intercellular adhesion molecule-1 on the graft endothelium, induced by ischaemia during organ procurement, may facilitate immune-cell recruitment into the graft and may partially explain the increased incidence of acute rejection in the setting of transplant acute tubular necrosis. A phase I study of monoclonal antibodies to intercellular adhesion molecules in patients at high risk of acute tubular necrosis showed that this agent decreased the incidence of acute rejection and reduced reperfusion injury in those patients with adequate concentrations

of circulating antibody.71 A randomised, multicentre, controlled trial with the same agent has not reproduced these findings (personal communication: CB Carpenter).

Antibodies directed against leucocyte function antigen-1 (LFA-1), a ligand for intercellular adhesion molecule-1, may provide similar prophylaxis against acute rejection to antilymphocyte globulin when given in the early post-transplant period.72 Anti-LFA-1 monoclonal antibodies also protect against delayed graft function. A multicentre trial of the effects of anti-LFA-1 monoclonal antibody therapy in cadaveric renal transplantation is in progress.

Blockade of T cell accessory molecules

Monoclonal antibodies directed against the CD4 molecule inhibit T cell activation by steric disruption of formation of complexes between T cell receptor and major histocompatibility complex peptides. In addition, some antibodies induce depletion of circulating CD4 T cells and others may modulate signalling through the CD4 molecule. In phase I and phase II clinical studies, monoclonal antibodies to CD4 administered early after transplantation with standard triple-therapy immunosuppression were well tolerated, but did not reduce the incidence of acute rejection.73,74 The latter finding may be a consequence of reduced efficacy owing to recipient production of antimurine antibodies. However, chimeric or humanised anti-CD4 antibodies do not seem to be undergoing further clinical development at this time.

The ligand for T cell CD2 is LFA3, a molecule expressed by a variety of cell types including antigen-presenting cells and endothelial cells.75 Endothelial LFA3 may be an important activational stimulus for T cells as they migrate across the graft endothelium. LFA3TIP, a human fusion protein, has been developed to block CD2/LFA3 interactions and has been shown to extend cardiac allograft survival in a primate model.76 In a small randomised trial, the rodent IgG2b anti-human CD2 monoclonal antibody BTI-322 reduced the incidence of acute rejection and delayed graft function in patients receiving triple-therapy immunosuppression.77

CD45 is a protein-tyrosine phosphatase present on all leucocytes and is a critical regulator of T cell activation. Monoclonal antibodies directed against the CD45RB isoform extend renal graft survival in mice.78 More information is required about the biological function of the various CD45 isoforms before initiation of studies in human transplant recipients. Perfusion of human renal allografts with anti-CD45 monoclonal antibodies before transplantation has already been done in an attempt to deplete grafts of passenger leucocytes.79

Peptide-mediated immunosuppression

Peptides derived from class I and class II major-histocompatibility-complex molecules are known to have immunomodulatory effects in vitro and in vivo.80,81 Research has focused mainly on peptides derived from the a-1 helical region of class I major-histocompatibility-complex molecules, particularly residues 75-84 of the human HLA B7 and HLA B2702 alleles. In animal models, these peptides extend cardiac, small bowel, and skin allograft survival, either alone, or in combination with a subtherapeutic dose of cyclosporin.81 These peptides function, at least in part, through inhibition of recipient natural killer cell and cytotoxic T lymphocyte function. Peptides derived from the a helical region of class II major-histocompatibility-complex molecules inhibit CD4 T cell proliferation in vitro by targeting cellular processes similar to those affected by sirolimus. Why components of major-histocompatibility-complex molecules should exhibit such immunosuppressive effects is a fascinating question. These molecules probably have important physiological immunomodulatory roles in vivo.

A small, randomised, double-blind (phase II) trial to assess the safety and pharmacokinetics of the HLA-B27.85-84 peptide in recipients of a first cadaveric renal allograft was published in 1997.82 Patients received standard triple-therapy immunosuppression and variable doses of peptide or placebo for the first 10 days after transplantation. There was no

difference in the incidence of acute rejection between groups. Patients receiving the highest dose of peptides showed significant impairment of natural killer cell function for up to 2 months, but the biological significance of this finding is undefined. Further work with both class I and class II major-histocompatibility-complex peptides is anticipated.

Gene therapy in transplantation

Major advances have been made in techniques to deliver genetic material into cells. Experimentally, these techniques have been useful in the dissection of the immunobiology of transplant rejection. In clinical transplantation, gene therapy may enable immunomodulatory agents to be expressed specifically within the graft, thereby overcoming the difficulties of systemic immunosuppression.83 The ability to alter the genetic make-up of animals such as pigs may eventually result in successful xenotransplantation.84

Tolerance induction

The ultimate goal in transplantation is the development of tolerance, defined as donor-specific unresponsiveness without the need for combined immunosuppression.85 Since the pioneering work of Medawar in the 1950s, substantial research has been directed towards the induction of tolerance in experimental models of transplantation. Although the precise mechanisms are not understood, most strategies devised to induce tolerance involve a combination of pretreating the recipient with donor antigen, and short courses of immunosuppressive or immunomodulatory therapy. Tolerance induction requires that alloreactive T cell clones are rendered unreactive. This may be achieved through clonal anergy, activation-induced apoptosis, or induction of regulatory/suppressor cell function. Anergy and deletional mechanisms may occur within the thymus (central tolerance) or in the peripheral immune system (peripheral tolerance), whereas regulatory cells typically work in the periphery. Research into the mechanisms of activation-induced apoptosis has highlighted the importance of T cell interleukin-2 production in permitting apoptosis.86 Thus, interleukin-2 has a dual function -- it is essential for T cell proliferation but is also required for cell death. These findings may explain why calcineurin inhibitors, in particular cyclosporin, prevent the development of tolerance in some experimental models.87

Conclusion

Immunosuppressive therapy is undergoing an exciting period of change as increasing numbers of drugs make the transition from the laboratory bench to the clinical arena. Large multicentre trials have already shown that the incorporation of agents such as antibodies to interleukin-2 receptor, tacrolimus, or mycophenolate mofetil into immunosuppressive regimens significantly lowers the incidence of acute allograft rejection. Several important issues have yet to be addressed. First, are these agents providing more specific or simply more potent immunosuppression? More potent immunosuppression may lead to an unacceptable increase in long-term complications, such as neoplasia. Second, how can these newer agents best be combined to achieve maximum efficacy and to keep side-effects to a minimum? For example, the combination of mycophenolate mofetil with tacrolimus may prove to lower acute rejection rates further while reducing the risk of post-transplant diabetes mellitus (through lower steroid use). The capacity of interleukin-2 receptor blockade added to mycophenolate mofetil and steroids to obviate use of calcineurin inhibitors is being assessed. Certainly, the increased choice of immunosuppressive agents now allows greater flexibility in prescriptions for the individual transplant recipient. Third, will newer agents prevent chronic rejection and improve long-term graft survival? Given that acute rejection is an important risk factor for chronic rejection, it is hoped that reduction of its incidence will translate into better long-term graft survival. However, this hypothesis has yet to be proven in prospective clinical trials. Finally, the issue of cost-effectiveness cannot be ignored. Is the routine use of newer, more expensive drugs justified if evidence of improved long-term outcome is lacking? To address these issues will require major efforts in

the future from the transplant community. Design of adequate extended follow-up studies is particularly relevant, but cost and the pressures of the marketplace are major obstacles in this regard. In the long-term, the hope is that novel immunosuppressive strategies involving blockade of T cell costimulation, antigen recognition, and accessory molecule function will modulate the immune response such that long-term allograft acceptance in the absence of non-specific immunosuppression becomes a clinical reality.

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7/7/11 (Item 6 from file: 149)
DIALOG(R)File 149:TGG Health&Wellness DB(SM)

01794944 SUPPLIER NUMBER: 21176290 (THIS IS THE FULL TEXT)
Eradication of human T-lymphotropic virus type 1 by allogeneic bone- marrow transplantation.(Research Letters)
The Lancet, v352, n9133, p1034(1)
Sept 26,
1998

TEXT:

A boy aged 15 years with congenital pure red-cell anaemia and previously infected with human T-lymphotropic virus type 1 (HTLV-1) underwent allogeneic bone-marrow transplantation from his HLA-identical, HTLV-1-negative sibling. Engraftment was confirmed on day 28. His serum became HTLV-1 negative on day 320. PCR products for the HTLV-1 sequences gradually disappeared in blood and bone-marrow samples, and became undetectable 60 months after transplantation. Bone-marrow transplant therefore seems to be involved in HTLV-1 eradication.

This boy was diagnosed as having congenital pure red-cell anaemia after birth. He received several blood transfusions from his father who developed myelopathy in 1976, which was diagnosed as being associated with

HTLV-1 in 1980. At age 10 years, in 1982 the patient was identified as an HTLV-1 carrier. His mother was also positive for

HTLV-1 antibodies, but his three siblings were negative. At the time of bone marrow transplant the boy had no disease associated with HTLV-1, but had haemochromatosis, insulin-dependent diabetes mellitus, and chronic hepatitis C as a result of the blood transfusions. Busulfan and cyclophosphamide were used for preconditioning before transplantation . On Sept 9, 1992, he received bone marrow from his HLA-matched sister. Although he developed haemorrhagic cystitis, congestive heart failure, and convulsions, these were all well controlled. Leucocyte count rose to 1000/mL on day 22, and reticulocyte count by more than 1% (in 10000) by day 28. Platelet count remained low and anaemia persisted. On day 43, PCR for variable-number tandem repeat showed mixed chimerism , but it reverted to full chimerism after day 120.

The antibody to HTLV-1 was determined by gelatin particle agglutination assay (Serodia HTLV-1 kit, Fujirebio) and western blot (Problot HTLV-1 kit, Fujirebio).1,2 HTLV-1 genome was detected by nested PCR with primers amplifying the HTLV-1 pX, LTR, gag-pol, and env regions, as previously reported.3,4 Before transplantation, the antibody titre was 332 and western blot was positive. After transplantation, the antibody titre became negative on day 320. Western blot changes to soon after transplantation. In PCR analysis, all blood and bone-marrow samples were positive in all regions. After transplantation, blood samples remained positive in the pX region 15 months after transplantation (table). After 29 months, HTLV-1 genome was undetectable in blood samples. At 60 months after transplantation we confirmed complete negativity for HTLV-1 genome in blood and bone-marrow samples.

Some reports of allogeneic bone-marrow transplant in adult T-cell leukaemia suggest possible eradication of the malignant clone. In one report, however, HTLV-1 antibody still presented at 23 months after transplantation.5 Although our patient was a carrier of HTLV-1, and HTLV-1 carriage is associated with a 2-5% lifetime risk of developing a severe disease, we clearly showed that myelo-lymphoid-ablative chemotherapy and allogeneic bone-marrow transplant led to complete eradication of HTLV-1. This success may imply alternative strategies for the treatment of some other persistent viral infections.

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Department of Paediatrics, Osaka Medical Centre and Research Institute for Maternal and Child Health (K Kawa); and Sionogi Institute for Medical Science

01789528 SUPPLIER NUMBER: 21048706 (THIS IS THE FULL TEXT)
Prolonged survival of rat islet and skin xenografts in mice treated with donor splenocytes and anti-CD154 monoclonal antibody.
Gordon, Ethel J.; Markees, Thomas G.; Phillips, Nancy E.; Noelle, Randolph J.; Shultz, Leonard D.; Mordes, John P.; Rossini, Aldo A.; Greiner, Dale L.
Diabetes, v47, n8, p1199(8)
August,
1998

TEXT:

Treatment of C57BL/6 mice with one transfusion of BALB/c spleen cells and a brief course of anti-CD154 (anti-CD40 ligand) antibody permits BALB/c islet grafts to survive indefinitely and BALB/c skin grafts to survive for ~50 days without further intervention. We now report adaptation of this protocol to the transplantation of islet and skin xenografts. We observed prolonged survival of rat islet xenografts in mice treated with donor-specific spleen cell transfusion and anti-CD154 monoclonal antibody (mAb). Challenge islet xenografts placed on these animals indicated that graft acceptance was species-specific but not strain specific. Spleen cells from recipients bearing intact grafts led to rejection of rat islet xenografts in scid mice, suggesting that graft acceptance was not due to complete clonal deletion of xenoreactive cells. We also observed prolonged survival of rat skin xenografts in mice treated with donor-specific transfusion and anti-CD154 mAb. Prolonged survival of skin xenografts was also species specific. We conclude that treatment with appropriately timed donor-specific transfusion and anti-CD154 mAb induces durable survival of both islet and skin xenografts in mice. Because this procedure is targeted directly at CD154, a co-activation molecule expressed predominantly by activated (CD4.sup.+) T-cells, the results suggest that (CD4.sup.+) cells have a major role in the cellular immune response to xenografts. Diabetes 47:1199-1206, 1998

Allotransplantation to cure human diabetes faces the obstacle of obtaining adequate tissue from human donor pancreata. In the U.S. alone, an estimated 8 million people have been diagnosed with diabetes, and an equal number of individuals are thought to have undiagnosed disease (1). Obtaining adequate numbers of allografts may become an insurmountable problem. The United Network for Organ Sharing scientific registry reported in 1995 that its organ recipient waiting list had grown to approximately 38,000 individuals (2), and an estimated 9 people die each day awaiting a human allograft. An alternative to the human allograft is the xenograft (3-7). Once thought an unlikely modality for treating human disease, experimental transplants of baboon hearts, baboon bone marrow, and porcine neural tissue and islets in humans have already been attempted (2,8).

Those attempts at xenotransplantation have generally employed prolonged immunosuppression. A more attractive option is the induction of immunologic tolerance to xenografts (3,9-13). Several tolerance induction strategies have been applied to rat-to-mouse and human-to-mouse islet xenografts (2,3). These strategies have sought to alter donor islet tissue antigenicity, to modulate the host immune response, or both.

Attempts to reduce the antigenicity of transplanted islets have used in vitro culture to remove graft antigen-presenting cells (APCs). Other strategies have used anti-major histocompatibility complex (MHC) class II monoclonal antibody (mAb) to remove islet APCs or anti-MHC class I mAb to mask donor islet antigens (2,3).

Strategies for modulating the host response have included the use of CTLA4 -Ig to block co-stimulatory signals (14), treatment with anti-lymphocyte serum (15), depletion of host (CD4.sup.+) and/or (CD8.sup.+) T-cells (16-18), and the intrathymic injection of xenogeneic islet and bone marrow cells (19). Another strategy has been the creation of xenogeneic bone marrow chimeras in advance of subsequent islet transplant (20). None of these strategies has yet been applied to human islet xenotransplantation in the clinic (2,3).

We report here a novel strategy for the modification of the host immune response to xenografts. We have previously demonstrated the induction of long-term survival of murine islet allografts by pretreatment of recipients with a donor-specific transfusion (DST) of splenocytes and injections of anti-CD154 mAb directed against the CD40 ligand (21,22). This form of combination therapy also prolongs the survival of murine skin allografts, with 20% of grafts surviving (is greater than) 100 days (23). In a preliminary communication, we showed that this method of prolonging allograft survival can be extended to LEW rat islet and skin xenografts transplanted to C57BL/6 mice (24). The present study documents that the combination of DST and anti-CD154 mAb induces species-specific acceptance of rat islet xenografts in mice. We also document that this protocol is effective in a much more stringent skin transplantation system, suggesting that the protocol is robust and may be applicable to tissues other than islets.

RESEARCH DESIGN AND METHODS

Animals. C57BL/6 ((H-2.sup.b)) and BALB/c ((H-2.sup.d)) mice were obtained from the National Cancer Institute (Frederick, MD). C57BL/(6-prkdc.sup.scid)/(prkdc.sup.scid) mice ((H-2.sup.b), hereafter referred to as C57BL/6-scid mice) were obtained from The Jackson Laboratory (Bar Harbor, ME). DR-BB/Wor rats ((RT-1.sup.u)) were obtained from the colony sponsored by the National Institutes of Health at the University of Massachusetts Medical Center, Worcester, MA. Lewis (LEW) rats ((RT-1.sup.l)) were obtained from Harlan Sprague Dawley (Indianapolis, IN). All animals were housed under specific pathogen-free conditions in sterile cages with micro-isolator lids and given autoclaved food anti water ad libitum. Rats and mice used in these studies were certified to be serologically free of Sendal virus, pneumonia virus of mice, sialodacryoadenitis virus, rat corona virus, Kilham rat virus, H1 (Toolan's virus), GD7, Reo-3, lymphocytic choriomeningitis virus, mouse adenovirus, Hantaan virus, Mycoplasma pulmonis, and encephalitozoon cuniculi. All animals were maintained in accordance with recommendations in the U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training, the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council, National Academy of Sciences, 1996) and local institutional guidelines.

Antibodies and flow microfluorimetry. Phycoerythrin (PE)-conjugated mouse anti-rat CD45 (clone OX-1, pan-lymphohemopoietic cells), FITC-conjugated rat anti-mouse (H-2 K.sup.b) (AF6-88-5, anti-MHC class I), biotinylated hamster anti-mouse CD3 (145-2C11, pan T-cells), PE-conjugated rat anti-mouse CD-19 (1D3, pan B-cells), and PE-mouse anti-rat CD45R (HIS24, pan B-cells) mAb were obtained from Pharmingen (San Diego, CA). Cy-Chrome streptavidin, used for the visualization of bound biotinylated anti-mouse CD3 mAb, was obtained from Pharmingen. Anti-rat CD45 anti anti-mouse (H-2 K.sup.b) were used in dual-label studies to examine the level of rat cell chimerism in DST and anti-CD154 mAb-treated recipients. The level of detection of rat DR-BB/Wor splenocytes in the presence of mouse C57BL/6 splenocytes was determined to be 0.5% in independent mixing experiments (data not shown). Flow microfluorimetry was also used to determine the level of lymphocyte engraftment in C57BL/6-scid adoptive recipients of spleen cells from C57BL/6 donors.

Flow cytometry analysis was performed as described (25,26). Briefly, single cell suspensions were labeled with antibody, rinsed, fixed in 1% paraformaldehyde, and analyzed on a FACScan (Becton Dickinson, Sunnyvale, CA). Dead cells and erythrocytes were excluded by electronic gating. At least: (10.sup.4) events were analyzed for each sample.

Pancreatic islet transplantation. C57BL/6 and C57BL/6-scid recipient mice 8-10 weeks of age were rendered hyperglycemic by a single intraperitoneal injection of 150 or 160 mg/kg of streptozotocin, respectively. Plasma glucose was measured using a Beckman II glucose analyzer (Beckman, Fuller(on, CA). The presence of diabetes in graft

recipients was established by the observation of a plasma glucose concentration (is greater than) 250 mg/dl on at least two different days before transplantation. Pancreatic islets were harvested from donor rats or mice by collagenase digestion, as previously described (21,27). Islets were transplanted at a dose of 20/kg body wt into the right renal subcapsular space of the recipient. Islet donors were adult DR-BB/Wor rats of either sex, 8- to 9-week-old male LEW rats, or 4- to 6- month-old female BALB/c mice. Grafts were judged to have failed after the plasma glucose concentration rose to (is greater than) 250 mg/dl on 2 successive days. In the case of all islet transplant recipients that were normoglycemic at the end of the period of experimental observation, the function of the transplanted islets was confirmed by removal of the kidney containing the islet transplant and documentation of the return of the plasma glucose concentration to (is greater than) 250 mg/dl.

Skin transplantation. Skin-graft recipients were male C57BL/6 mice or male C57BL/6-scid mice 8-10 weeks of age. Full-thickness skin specimens for transplantation were obtained from 3- to 6-week-old DR-BB/Wor rats of either sex and 4- to 6-month-old female BALB/c mice that had been killed in 100% (CO.sub.2). Donated skin was prepared and transplanted using a modified (23) Billingham and Medawar procedure (28). Skin-graft survival was assessed three times weekly by visual and tactile examination. Time of rejection was defined as the first day on which the entire epidermal surface of the graft was necrotic.

Treatment of islet and skin xenograft recipients with DST and anti-CD154 mAb. DR-BB/Wor rats were killed in an atmosphere of 100% (CO.sub.2). Spleens were removed, mechanically dispersed in sterile medium (RPMI-1640), washed, and counted using a hemocytometer. Cells were assayed for viability using the method of Trypan blue exclusion, and in all cases viability was (is greater than) 90%. The MR1 hamster anti-mouse CD154 mAb was produced as ascites in scid mice (23,29,30). A standard enzyme-linked immunosorbent assay for hamster Ig measurement was used to determine anti-CD154 mAb concentration in ascites. Anti-CD154 ascites were diluted to a concentration of 1 mg/ml and administered at doses of 0.25 mg/mouse. Islet and skin xenograft recipients received no treatment, a single transfusion of donor spleen cells, a single course of anti-CD154 mAb, or combined therapy with both reagents as previously described (21,23). DSTs varied from 5-50 x (10.sup.6) viable spleen cells in a volume of 0.2-0.4 ml given via the tail vein 5-7 days before grafting. Courses of anti-CD154 mAb comprising either 4 or 14 doses were given intraperitoneally twice weekly beginning on the (lay of spleen cell injection. Injections were generally given on Mondays and Thursdays.

Histology. Islet xenograft recipients whose grafts rejected were nephrectomized and then killed immediately. In the case of animals that were normoglycemic at the conclusion of an experimental protocol, nephrectomy of the kidney containing the islet graft was performed, and the animal was allowed to survive to confirm graft function. Kidneys into which grafts had been transplanted were fixed in Bouin's solution overnight, washed, and stored in 10% buffered formalin. Paraffin-embedded sections were prepared and stained with Masson trichrome; additional sections were stained immunohistochemically for the presence of insulin and glucagon. A qualified pathologist; who was unaware of the treatment status of the donors evaluated all islet graft specimens for the presence of inflammatory cells, insulin, and glucagon. Because no skin grafts survived indefinitely, none were studied histologically.

Statistical analysis. Average duration of graft survival is presented as the median. Statistical comparisons of graft survival among groups were performed using the method of Kaplan and Meier (31); the equality of xenograft survival distributions for animals in different treatment groups was tested using the log-rank statistic (32). P values (is less than) 0.05 were considered statistically significant.

RESULTS

Rat-to-mouse islet xenografts

Prolonged survival of rat islet xenografts in mice treated with DST and anti-CD154 mAb. We first tested the hypothesis that treatment with DST and anti-CD154 mAb would prolong rat islet xenograft survival in mice. Streptozotocin-diabetic C57BL/6 mice were randomized into four groups, each of which received a DR-BB/Wor rat islet graft placed beneath the renal capsule. Group 1 (n = 20) received no other treatment. Group 2 (n = 5) received a single transfusion of 5 x (10.sup.6) DR-BB/Wor rat spleen cells 5-7 days before transplantation. Group 3 (n = 15) was given anti-CD154 mAb (0.25 mg) twice weekly for 7 weeks beginning 5-7 days before transplantation. Group 4 (n = 29) received both anti-CD154 mAb and one transfusion of 5-25 x (10.sup.6) spleen cells, with the first dose of antibody being administered on the same day as the transfusion. Animals were observed until hyperglycemia recurred or until days 63-72 after transplantation, at which time unilateral nephrectomy was performed to document the function and histology of the islet xenograft. Hyperglycemia was observed to recur in all nephrectomized graft recipients.

The animals in group 4 received DST at doses of 5 x (10.sup.6) (n = 10), 10 x (10.sup.6) (n = 14), or 25 x (10.sup.6) (n = 5) cells. Median survival times (MSTs) associated with these doses were 71, 72, and 63 days, respectively. Life-table analysis showed that DST dose had no statistically significant effect on graft survival (P = 0.93), and these recipients are considered as one group in subsequent analyses.

Overall life-table analysis (Fig. 1) demonstrated that combined treatment with DST plus anti-CD154 mAb (group 4) was associated with significantly longer islet graft survival (MST = 71 days) than was treatment with anti-CD154 mAb alone (group 3, MST = 41 days, P (is less than) 0.05), DST alone (group 2, MST = 8 days, P (is less than) 0.001), or no treatment (group 1, MST = 11 days, P (is less than) 0.001). In the case of the recipients of combined therapy, 69% of islet grafts were still functioning when the treated animals were nephrectomized on days 62-73 and entered into additional experiments.

(Figure 1 ILLUSTRATION OMITTED)

Although not as effective as the combination of DST plus antibody, treatment with anti-CD154 mAb alone was statistically superior to treatment with DST alone (P (is less than) 0.001) or no treatment (P (is less than) 0.001). In contrast, treatment with DST alone in advance of the islet xenograft was associated with accelerated rejection of islets (MST - 8 days) when compared with rejection in untreated control recipients (MST = 11 days, P (is less than) 0.002).

Histological analysis of functioning xenogeneic islets obtained at the time of nephrectomy revealed the presence of readily detectable insulin- and glucagon-containing cells. Few, if any, mononuclear cells were observed within the islet grafts, but foci of inflammatory cells were observed at the periphery of the grafted tissue ("peri-insulitis," Fig. 2). No islet tissue was detectable in any of the grafts that had ceased to function (data not shown).

(Figure 2 ILLUSTRATION OMITTED)

Abbreviated courses of anti-CD154 mAb plus DST are less effective. In a separate experiment, diabetic C57BL/6 mice were given either no additional treatment (n = 11) or DST at a dose of 5 x (10.sup.6) cells plus anti-CD154 mAb 0.25 mg twice weekly for 2 weeks starting on the day of transfusion (n = 5). All mice were given DR-BB/Wor rat islet grafts; in the case of treated animals, grafts were placed 5-7 days after transfusion. Graft survival in the recipients of DST plus this abbreviated course of anti-CD154 mAb (MST = 20 days) was statistically superior to graft survival in controls (MST = 10 days, P (is less than) 0.05) but inferior to that achieved using 14 doses of antibody plus DST (group 4 in Fig. 1).

Challenge islet xenograft studies. The C57BL/6 mice described above that had been treated with one transfusion of 5-25 x (10.sup.6) DR-BB/Wor rat spleen cells and 14 doses of anti-CD 154 mAb, and whose DR-BB/Wor rat islet xenografts had survived 62-73 days, were entered into a challenge graft protocol. This protocol was designed to determine if xeno- or

alloreactive cells were still present in mice with successful islet xenografts. The mice first underwent unilateral nephrectomy of the kidney bearing the xenograft. After documentation of recurrent hyperglycemia for 7-14 days, the mice were randomized to receive either a second (RT1.sup.u) DR-BB/Wor rat islet xenograft (n = 12), an MHC-disparate (RT1.sup.1) LEW rat islet xenograft (n = 4), or a fully allogeneic H-(2.sup.d) BALB/c mouse islet graft (n = 3).

As shown in Table 1, 83% of DR-BB/Wor and 100% of LEW rat islet challenge grafts were accepted by their hosts and restored normoglycemia for a minimum of 36 days, whereas all mouse islet allografts were rejected 18-22 days after implantation. Six of the mice that tolerated their DR-BB/Wor islet grafts were entered into a third protocol (see below) on days 36-60 after challenge grafting. DR-BB/Wor grafts in the remaining four recipients were documented to function through 103 days after challenge. Recipients of LEW islet xenografts were followed for 95-111 days before being used in the third protocol.

(TABULAR DATA 1 NOT REPRODUCIBLE IN ASCII)

Adoptive transfer studies. In a final experiment, spleen cells were obtained from the C57BL/6 mice that 1) had been treated with DST and anti-CD154mAb, 2) had accepted a primary DR-BB/Wor rat islet xenograft, and 3) had then accepted either a. DR-BB/Wor or LEW islet challenge xenograft. The spleen cells (25 x (10.sup.6) were adoptively transferred to chemically diabetic C57BL/6-scid mice (n = 32) that had received a DR-BB/Wor rat islet xenograft 10-14 days earlier. Control C57BL/6-scid mice bearing DR-BB/Wor islet xenografts received either no treatment (n = 5) or a single transfusion of 25 x (10.sup.6) spleen cells obtained from normal, untreated C57BL/6 donors (n = 5).

As shown in Table 2, adoptive transfer of spleen cells from normal C57BL/6 donors was associated with prompt rejection (MST = 13 days after transfusion) of the DR-BB/Wor islet grafts in the scid recipients. Adoptive transfer of cells from the experimental mice was also associated with rejection of the DR-BB/Wor rats in the scid mice, but rejection was delayed (Table 2). Overall MSTs were 68 days in the case of spleen cells from mice bearing DR-BB/Wor challenge xenografts and 49 days in the case of spleen cells from mice bearing LEW challenge grafts. Control xenografts in C57BL/6 scid mice that were otherwise untreated remained functional through the end of the experiment (day 105).

TABLE 2

Survival of islet xenografts in scid mice after adoptive transfer of spleen cells

Anti-CD154 mAb	DST (x (10.sup.6))	Survival of primary DR-BB graft before challenge graft (days)
Yes	5	73
		72
		73
		71
Yes	10	73
		72
Yes	25	68
		66
		67
		68
Yes	None	71
		73
Yes	10	73
		73
No	None	No graft

Anti-CD154 mAb	Challenge graft donor and survival (days)	Graft survival in scid mice after cell transfer	Overall MST (days)
Yes	DR-BB, 36	83, 172	
	DR-BB, 36	25, 25	
	DR-BB, 36	25, 172	
	DR-BB, 40	12, 119	
Yes	DR-BB, 40	45, 87	
	DR-BB, 40	168, 168	
Yes	DR-BB, 60	41, 52	
	DR-BB, 60	20, 15	
	DR-BB, 60	>94, 52	
	DR-BB, 60	>94, >94	
Yes	LEW, 110	8, 27	
	LEW, 110	13, 13	
Yes	LEW, 110	13, 71, >105	
	LEW, 110	27, >97	
	LEW, 111	>105, >105, >105	
No	--	8, 8, 13, 13, 42	
Yes			
Yes			68
Yes			
Yes			
Yes			49
No			13

Adoptive recipients were chemically diabetic C57BL/6-scid mice that had been successfully transplanted with DR-BB rat islet grafts 10-14 days earlier. Spleen cell donors were C57BL/6 mice that had received anti-CD154 mAb and one transfusion of DR-BB rat spleen cells, had borne primary DR-BB rat islet xenografts for 66-73 days, and had borne challenge DR-BB or LEW rat islet xenografts for 36-111 days. All donors were normoglycemic at the time spleen cells were obtained. Each graft-bearing C57BL/6-scid recipient received 25 x (10.sup.6) spleen cells. Islet graft survival relative to the day of adoptive transfer is shown. Except; for the controls given a transfusion from normal untreated C57BL/6 donors, each line in the table presents data from an individual spleen cell donor. Overall MST for each related group of donors is shown in the far right column and was 68 days for spleen cells from mice bearing DR-BB/Wor challenge xenografts and 49 days for spleen cells from mice bearing LEW challenge grafts. The C57BL/6-scid mice bearing DR-BB rat islet grafts but given no spleen cells remained normoglycemic for the duration of the experiment (105 days, n = 5).

Rat-to-mouse skin xenografts Prolonged survival of rat skin xenografts in mice treated with appropriately timed DST and anti-CD154 mAb. We next tested the hypothesis that treatment with DST and anti-CD154 mAb treatment would also prolong rat, skin xenograft survival in mice. C57BL/6 mice were randomized into 6 groups, each of which received a DR-BB/Wor rat skin graft. Group 1 (n = 18) received no other treatment. Groups 2 and 3 were given anti-CD154 mAb (0.25 mg) twice weekly for 7 weeks beginning 7 (group 2, n = 9) or 14 (group 3, n = 6) days before transplantation. Groups 4 and 5 received both anti-CD154 mAb using the same dosing schedule and one

transfusion of 10 x (10.sup.6) spleen cells; the transfusion and the first dose of antibody were administered either 14 days before (group 4, n = 5), or on the day of transplantation (group 5, n = 7). group 6 (n = 25) received both anti-CD154 mAb and one transfusion of 5 x (10.sup.6) or 10 x (10.sup.6) spleen cells, with the transfusion and the first dose of antibody being administered 5-7 days before transplantation.

The animals in group 6 received DST at doses of 5 x (10.sup.6) (n = 11) or 10 x (10.sup.6) (n = 14) cells. A preliminary life-table analysis showed that DST dose had no statistically significant effect on graft survival (P = 0.53), and these recipients are considered as one group in subsequent analyses.

Overall life-table analysis is shown in Fig. 3. Combined treatment with DST plus anti-CD154 mAb begun on day -7 (group 6) was associated with significantly longer skin xenograft survival (MST = 36 days) than was treatment with anti-CD154 mAb alone begun on day-7 or day-14 (groups 2 and 3, MST = 9 and 6 days, respectively, P (is less than) 0.001 in each case), combined treatment begun on day -14 (group 4, MST = 11 days, P (is less than) 0.02), or combined treatment begun on 0 (group 5, MST = 10 days, P (is less than) 0.001). No grafts in any group survived indefinitely.

(Figure 3 ILLUSTRATION OMITTED)

An abbreviated course of anti-CD154 mAb plus DST is comparably effective. We also tested the efficacy of combined treatment with DST and a briefer course of anti-CD154 mAb in prolonging rat skin xenograft survival in mice. C57BL/6 mice were randomized into three groups, each of which received a DR-BB/Wor rat skin graft. Group 1 (n = 15) received no other treatment. Group 2 (n = 15) was given anti-CD154 mAb (0.25 mg) twice weekly for 2 weeks beginning 7 days before transplantation. Group 3 (n = 25) received both anti-CD154 mAb on the same dosing schedule and one transfusion of 5-50 x (10.sup.6) spleen cells, with the transfusion being administered with the first dose of antibody.

The animals in group 3 received DST at doses of 5 x (10.sup.6) (n = 10), 25 x (10.sup.6) (n = 10), or 50 x (10.sup.6) (n= 5) cells. Life-table analysis showed that DST dose had no statistically significant effect on graft survival (P = 0.25), and these recipients are considered as one group in subsequent analyses.

As shown in Fig. 4, the combination of DST plus four doses of anti-CD154 mAb (group 3) prolonged skin xenograft survival (MST = 29 days) compared with either no treatment (group 1, MST = 7 days, P (is less than) 0.01) or anti-CD154 mAb treatment alone (group 2, MST = 7 days, P (is less than) 0.01). The degree of prolongation achieved with DST plus the abbreviated (4-injection) course of anti-CD154 mAb treatment (MST = 29 days, group 3 in Fig. 4) was statistically similar (P = 0.17) to that achieved using the standard 14-injection regimen (MST = 36 days, group 6 in Fig. 3).

(Figure 4 ILLUSTRATION OMITTED)

Challenge skin xenograft studies. The C57BL/6 mice described above that had been treated with one transfusion of 5-25 x (10.sup.6) DR-BB/Wor rat spleen cells and 14 doses of anti-CD154 mAb, and whose DR-BB/Wor rat skin xenografts had survived 50-70 days, were entered into a challenge graft protocol. This protocol was designed to determine if xeno- or alloreactive cells were still present in mice at a time when skin xenografts were healed and free of any sign of rejection. The mice were randomized to receive either a second (RT1.sup.u) DRBB/Wor rat skin xenograft (n = 5) or a fully allogeneic H-(2.sup.d)BALB/c mouse islet graft (n = 2).

As shown in Table 3, 100% of DR-BB/Wor challenge skin xenografts were eventually rejected by their hosts. Rejection of the challenge grafts correlated temporally with rejection of the primary xenografts; three of the five mice rejected both the challenge and primary grafts within 9 days of challenge. In contrast, the remaining two mice maintained both the challenge and the primary grafts for 34-41 days. The total duration of primary skin xenograft survival in these two mice was 111 days.

TABLE 3
Survival of primary skin xenografts after challenge with a second skin graft

Treatment of recipient primary skin graft	Duration of primary skin graft survival at the time of challenge grafting (days)
DST (5 x (10.sup.6)), anti-CD154 mAb	70
	70
DST (10 x (10.sup.6)), anti-CD154 mAb	70
	70
DST (25 x (10.sup.6)), anti-CD154 mAb	50
	50

Treatment of recipient primary skin graft	Challenge graft
DST (5 x (10.sup.6)), anti-CD154 mAb	DR-BB rat
	DR-BB rat
DST (10 x (10.sup.6)), anti-CD154 mAb	DR-BB rat
	DR-BB rat
DST (25 x (10.sup.6)), anti-CD154 mAb	BALB/c mouse
	BALB/c mouse

Treatment of recipient primary skin graft	Challenge graft	Primary graft
	8	9
DST (5 x (10.sup.6)), anti-CD154 mAb	36	41
	8	8
DST (10 x (10.sup.6)), anti-CD154 mAb	8	9
	34	41
	9	9
DST (25 x (10.sup.6)), anti-CD154 mAb	9	9

C57BL/6 mice that had been treated with a DST and 14 doses of anti-CD154 mAb and whose DR-BB/Wor rat skin xenografts had survived 50-70 days received the challenge grafts indicated in the table.

The two C57BL/6 recipients that had borne DR-BB rat skin xenografts for 50 days and were then challenged with allogeneic BALB/c skin rejected both the challenge allograft and the primary xenograft within 9 days of challenge (Table 3).

Survival of C57BL/6 mouse skin xenografts on DRBB/Wor rats is not prolonged by treatment with C57BL/6 mouse DST and anti-mouse CD154 mAb. In our rat-to-mouse transplantation protocol, treatment with anti-CD154 mAb could have mediated its effect on CD (154.sup.+) cells present in the recipient, (CD (154.sup.+) cells present in the DST, or both. To help discriminate among these possibilities, reciprocal mouse-to-rat skin xenografts were performed. DR-BB rats were given no treatment (n = 9), hamster anti-mouse CD154 mAb (n = 10), or the combination of anti-CD154 mAb and one transfusion of 20 x (10.sup.6) C57BL/6 mouse spleen cells (n = 9). The first dose of antibody was administered on the same day as the transfusion, 5-7 days before transplantation. Antibody treatment was continued biweekly throughout the experiment. Rejection of skin xenografts occurred rapidly in all three groups (MST = 6, 7, and 10 days, respectively).

DISCUSSION

These data document that the combination of a single transfusion of donor spleen cells and a brief course of anti-CD154 mAb markedly prolongs the survival of concordant rat islet xenografts in mice. More than 75% of islet grafts were still functional 70 or more days after implantation. At that point, the grafts were removed for studies of the mechanism of

tolerance induction, but given our published preliminary observations (24), it is likely that most of these grafts could have survived much longer. In that preliminary study, which used a very similar protocol, 6 of 9 rat islet xenografts were still functional 175 days after transplantation. Taken together, these two data sets suggest that combined treatment with DST and anti-CD154 mAb induces durable islet xenograft survival.

In the present study, the combination of DST and anti-CD154 mAb also prolonged the survival of rat skin xenografts. Some skin xenografts survived (is greater than) 100 days, a remarkable result given the strong immunogenicity of skin. The outcome of the challenge graft studies suggests that, in some treated animals, levels of xenoreactive cells are low for long periods of time.

Skin xenograft survival in the present study is superior to that reported in our preliminary communication (24). In that study, the DST was manipulated to remove fibronectin adherent splenocytes. The superior outcome in the present study may reflect both the use of nonmanipulated spleen cells for the DST and refinement of our technique. Based on theoretical considerations, our original implementation of the two-element protocol used elutriated small B-cells (21). Subsequent work has demonstrated, however, that unfractionated spleen cells are equally efficacious in prolonging allograft survival (23,33), and the present study extends this observation to the xenograft system. Studies to identify those specific cell populations that mediate the effect of DST in our two-element protocol are in progress in our laboratory.

It is worth noting that the outcomes for both skin and islet grafts were in part dependent on the dosage and timing of treatment, suggesting that additional refinement of the protocol may further improve outcomes.

The present data also suggest that the prolongation of xenograft survival induced by DST and anti-CD154 mAb is species- but not strain-specific. Mice that had accepted DRBB rat xenografts would accept both DR-BB and LEW challenge xenografts, but not BALB/c mouse challenge allografts. These observations are consistent with previous reports of species-specific xenotolerance induced by the combination of islet culture, intrathymic injection of islets, and treatment of the host with anti-lymphocyte serum (34).

Our histological findings in xenogeneic islets are also consistent with previous observations (34). It appears that xenografted islets continue to function despite the presence of focal mononuclear cell infiltrates near the islets. The identity and functional characteristics of these infiltrating cells are unknown and the subject of ongoing study in our laboratory.

In the present study, we observed that treatment with anti-CD 154mAb alone was somewhat effective in prolonging the survival of islet xenografts, but completely ineffective when applied to skin xenografts. This observation is consistent with our earlier reports that anti-CD 154 mAb by itself can prolong the survival of allogeneic islet (21, 22) but not skin (23) grafts. The data suggest the possibility that there could exist co-stimulatory pathways in skin that are not dependent on the interaction of CD40 with CD154. Such pathways might be mediated by APCs found in skin but not islets, perhaps epidermal Langerhans cells. The data we have obtained using anti-CD154 mAb alone in both allograft and xenograft systems are consistent with this interpretation and suggest that such co-stimulatory activity may be much more robust in skin than in islet tissue. More generally, the data suggest that prolongation of xenograft survival using anti-CD154 mAb alone is likely to be achievable only in favorable species using only favorable tissues.

The present data also provide additional insight into the nature of the immune response to xenogeneic tissues. The cellular immune response to xenografts is known to be distinct from the cellular immune response to allografts (7). Studies of xenograft rejection suggest that MHC class II is the predominant antigenic target and that MHC class II-restricted (CD4.sup.+) T-cells are the primary mediators of graft destruction (16-18).

It has been suggested, in fact, that xenograft rejection may use a (CD4.sup.+), cytotoxic T-cell-independent pathway (3,35). Lafferty and colleagues (35-37) have proposed the concept of "indirect" rejection based on the interaction of recipient (CD4.sup.+) T-cells with recipient APCs presenting xenoantigens. The subsequent release of cytokines and free radicals in the vicinity of grafted islets may then lead to (Beta)-cell cytotoxicity or apoptosis. Studies of cytokine gene expression are consistent with this view. For example, Morris et al. (38), studying the rejection of xenogeneic porcine proislets in CBA/H mice, have observed that Th2-like (CD4.sup.+) T-cells appear to be differentially activated by exposure to xenoantigen.

The results of the present study support the hypothesis that (CD4.sup.+) cells may, in fact, play a major role in the cellular immune response to xenografts. This interpretation is based on the fact that the protocol used in the present study is targeted directly at CD154, a co-activation molecule that is expressed predominantly by activated (CD4.sup.+) T-cells (39, 40).

Our working hypothesis for the prolongation of xenograft survival by DST and anti-CD154 mAb is that foreign antigen presented in the absence of co-stimulation (which is blocked by anti-CD154 mAb) leads to T-cell nonresponsiveness. When applied to the induction of long-term allograft survival, the anti-CD154 mAb component of the protocol could be exerting its effect on either donor or recipient cells. In the rat-tomouse xenograft system we have studied, it is clear that the effect of the anti-mouse CD154 mAb is directed at host (CD154.sup.+) cells. In our reciprocal study of mouse-to-rat skin xenografts, we further observed that tolerance could not be induced in rat recipients of mouse xenografts, mouse DST, and anti-mouse CD154 mAb. These data suggest that neither "passenger" nor DST (CD154.sup.+) cells of donor origin are required for xenograft rejection; they are consistent with the view that "indirect" antigen presentation may mediate the rejection of xenogeneic tissues (35-37).

We conclude that combination therapy with DST plus antiCD40L mAb in mice can induce durable survival of concordant rat islet xenografts and can prolong the survival of rat skin xenografts. This treatment strategy is of particular interest because it affects only cells that are activated by antigen to express CD154, avoiding the potential complications of therapies that lead to generalized immunosuppression.

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APC, antigen-presenting cell; DST, donor-specific transfusion; mAb, monoclonal antibody; MHC, major histocompatibility complex; MST, median survival time.

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Differential T cell costimulatory requirements in CD28-deficient mice.
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TEXT:

The molecular basis of T cell costimulation is not fully understood but may involve ligands and soluble factors provided by antigen-presenting cells (APCs) that interact with specific T cell surface molecules (1-3). One major costimulatory pathway is characterized by the activation of the CD28 receptor (4, 5). Cross-linking of CD28 synergizes with T cell receptor (TCR) signals during T cell activation and can prevent the induction of T cell unresponsiveness in vitro (6). CD28 and the related CTLA-4 molecule (7) share a common ligand (B7), which is expressed on APCs (4, 7-9). In the mouse, CD28 molecules are constitutively expressed on almost all [CD4.sup.+] and [CD8.sup.+] peripheral T cells (10), whereas CTLA-4 is found on activated T cells only (11). Murine double positive ([CD4.sup.+][CD8.sup.+]) thymocytes express large amounts of CD28, whereas immature double negative ([CD4.sup.-][CD8.sup.-]) and mature single positive thymocytes have less CD28 (10). The B7 molecule, constitutively expressed on dendritic cells (12), is also expressed on other APCs and B cells and is up-regulated after activation (9, 12-14). Studies in vitro indicate that CD28-B7 engagement in combination with TCR occupancy delivers important signals for induction of proliferation of [CD4.sup.+] T cells and for T cell-dependent B cell differentiation (15).

The signaling pathways for CD28 have not yet been unraveled; however, tyrosine phosphorylation of cellular proteins changes after CD28 cross-linking (16). At transcriptional and posttranscriptional levels, CD28 can synergize with TCR signals (17, 18). Signals mediated by CD28 stabilize a set of important mRNAs for cytokines and monokines (17). Disruption of CD28 and CTLA-4 interactions with B7 by the introduction of a soluble form of CTLA-4 (CTLA4Ig) impedes cell-mediated immune responses in vitro (19) and leads to T cell unresponsiveness (6, 20, 21). Experiments in vivo suggest that treatment with CTLA4Ig can reduce IgG1 production in a primary response to keyhole limpet hemocyanin or sheep red blood cells (22). Similar treatment also results in long-term acceptance of xenogenic pancreatic islet grafts (23) and prolongs the survival of cardiac allografts (24). Transfection of B7 into tumor cells induces or augments the generation of a cell-mediated immune response to otherwise nonimmunogenic tumor cells (25, 26).

Despite such data, it has not been determined whether CD28 is the only costimulatory signal for T cell activation in vivo. The role of CD28 in viral infections and thymocyte differentiation and its interdependence with CTLA-4 have not yet been elucidated. To address these questions, we have established a mutant mouse strain lacking the CD28 costimulatory molecule.

The CD28 gene (27) was disrupted by a partial replacement of exon 2 with a neomycin resistance gene cassette (28) (Fig. IA). Homologous

recombination events were screened by polymerase chain reaction (PCR) and verified by Southern (DNA) blot analysis (Fig. 1B). Of the 814 colonies we screened that were G418-resistant, 3 contained the desired mutation. We obtained chimeric mice by injecting mutant embryonic stem cells into C57BL/6 blastocysts (29, 30). We tested for transmission of the mutated allele by mating the chimeric mice with (C57BL/6 x DBA/2)[F.sub.1] mice. Heterozygous offspring were intercrossed to generate mice homozygous for the targeted mutation of the CD28 gene. Homozygous mice were healthy and fertile, and we did not detect gross abnormalities in body weight, organ size, or number of lymphocytes in primary and secondary lymphatic organs. To verify inactivation of the CD28 molecule, we stained the cell surface with phycoerythrin (PE)-conjugated hamster monoclonal antibodies (mAbs) to mouse CD28 (Fig. 2A). Peripheral blood lymphocytes (PBLs) from homozygous targeted mice did not express CD28 molecules, indicating that the CD28 locus was disrupted in [CD28.sup.-/-] mice. The PBLs from heterozygous ([CD28.sup.-/-]) mice had decreased expression of cell surface CD28 (Fig. 2A). Thymocyte development in CD28-deficient mice appeared normal, as we judged from the expression of CD4, CD8 (Fig. 2B), CD3, heat-stable antigen, and interleukin-2 receptor [Alpha] (IL-2R[Alpha]) (31).

Analysis of the [V.sub.[Beta]] repertoire of CD28-deficient mice showed no defect in the clonal deletion of potentially self-reactive T cells. Mls-[1.sup.a]-reactive (32) [V.sub.[Beta]][6.sup.+] T cells were efficiently deleted in the peripheral blood of [CD28.sup.-/-] mice (0.2% [V.sub.[Beta]][6.sup.+] in [CD28.sup.-/-] Mls-[1.sup.a+] versus 7.5% [V.sub.[Beta]][6.sup.+] in [CD28.sup.-/-] Mls-[1.sup.a-]). Accordingly, [V.sup.[Beta]11-bearing T cells (33) were also depleted in CD28-deficient mice when I-E and endogenous viral products were present ([CD28.sup.-/-], [I-E.sup.+], 0.4% [V.sup.[Beta]][11.sup.4+]cells; [CD28.sup.-/-], [I-E.sup.-], 4.7% [V.sup.[Beta]][11.sup.+] cells). Splenic B cells were observed in normal numbers, and cell surface expression of B7 was not detectable in homozygous [CD28.sup.-/-] animals (Fig. 2B). Staining of splenocytes with mAb against CD3, TCR[Alpha][Beta], TCR[Gamma][Delta], and IL-2 receptor also did not reveal any significant alterations between littermate controls and [CD28.sup.-/-] mice (31).

Basal immunoglobulin (Ig) concentrations in [CD28.sup.-/-] mice were only 20% of those of wild-type littermates (Fig. 3). Analysis of Ig isotypes revealed a decrease in IgG1 and IgG2b, whereas IgG2a was increased significantly. Basal IgM and IgG3 titers seemed to be unaffected by the CD28 mutation (Fig. 3).

To evaluate the role of the CD28 molecule on T cell proliferation and cytokine secretion, we stimulated splenocytes from wild-type, heterozygous, and homozygous mice with the T cell lectin concanavalin A (Con A) (Fig. 4A). T cells derived from [CD28.sup.-/-] mice had a significantly reduced proliferative response to Con A. However, [CD28.sup.-/-] cells were not impaired in their growth potential when activated by a combination of phorbol ester [phorbol 12-myristate 13-acetate (PMA)] and calcium ionophore (Fig. 4A). Supernatants of lectin-stimulated cultures contained reduced amounts of T cell growth factors, as we determined by a standard CTLL-2 assay (Fig. 4B). The amount of IL-2R[Alpha] expression was much lower on lectin-activated [CD28.sup.-/-] T cells than on T cells derived from wild-type littermate controls (Table 1). Addition of exogenous IL-2 or T cell-conditioned medium to lectin-stimulated cultures only partially restored the proliferative response and IL-2R[Alpha] expression of [CD28.sup.-/-] T cells (Fig. 4A and Table 1). These results indicate that lectin-driven T cell responses are critically dependent on a functional costimulatory signal through CD28 and that this signal cannot be replaced completely in vitro by IL-2. [TABULAR DATA 1 OMITTED]

We tested antiviral T and B cell responses by infecting mutant and wild-type mice with lymphocytic choriomeningitis virus (LCMV) or vesicular stomatitis virus (VSV). The [CD28.sup.-/-] mice mounted a normal anti-LCMV cytotoxic T cell (CTL) response in vivo (Fig. 5A). To confirm that the capacity of [CD28.sup.-/-] mice to elicit a LCMV-specific CTL response was

unaltered, we injected LCMV into the footpads of mice. The footpads subsequently showed a normal immunopathological swelling reaction (Fig. 5B). The early phase of this swelling reaction is mediated exclusively by [CD8.sup.+] CTLs (34, 35).

After infecting CD28-deficient mice with VSV, we found neutralizing anti-VSV IgM titers to be normal. However, the class switch to neutralizing antibodies of the IgG class (34), which is strictly T helper cell-dependent, was reduced (Fig. 6). Taken together, these data indicate that the generation of [CD8.sup.+] CTLs in response to LCMV is not dependent, on interactions involving CD28, whereas T cell-B cell collaboration appears to be dependent on a functional CD28-B7 costimulation.

We have established a mutant mouse strain deficient for the CD28 gene. Despite expression of CD28 on thymocytes and B7 on APCs in wild-type mice (9, 10, 13), development of T lymphocytes in the CD28 mutant mice appears normal, suggesting that this interaction is not essential for thymic maturation of T cells. The ability to delete potentially self-reactive T cell receptors for M1s-[1.sup.a] and I-E indicates that thymic negative selection of [CD28.sup.-/-] mice is not dependent on CD28-B7 signaling.

Lectins do not activate highly purified T cells in the absence of APCs (36), yet the molecular basis of this phenomenon is not completely understood. Our data that the interaction of B7 and CD28 is critical for the mitogenicity of T cell lectins are consistent with preliminary in vitro experiments that showed that CTLA4Ig could block Con A-induced proliferation in murine [CD28.sup.+/+] splenocytes. Lectin-stimulated [CD28.sup.-/-] splenocytes did not secrete T cell growth factors, suggesting that T cell-dependent lectins are operative through CD28 costimulation. This observation is in accordance with previous reports that CD28 costimulation plays a decisive role in the production of IL-2 (20, 37). Even in the presence of T cell-conditioned medium (31) or exogenous recombinant IL-2, the CD28 defect cannot be overcome. Hence, CD28 signal transduction is an essential prerequisite for lectin-induced T cell activation. In addition, heterozygous [CD28.sup.+/-] splenocytes show a constant reduction in their responses to Con A. The T cells derived from these mice display only about 50% of their CD28 molecules at the cell surface (Fig. 2). This suggests that even the amount of CD28 expressed plays a role in the regulation of T cell immune functions. The defects in mitogen responsiveness of [CD28.sup.+/-] and [CD28.sup.-/-] T cells do not appear to be due to an intrinsic functional abnormality in these T cells, because [CD28.sup.+/+], [CD28.sup.+/-], and [CD28.sup.-/-] T cells responded equivalently to the chemical mitogens PMA and [Ca.sup.2+] ionophore, an activation scheme that bypasses the normal T cell activation pathways that are regulated by cell surface receptors.

In mice, CD28 is expressed on virtually all [CD4.sup.+] and [CD8.sup.+] T cells (10). It has been reported that the induction of [CD8.sup.+] CTL responses to tumors depends completely on the interaction of CD28 and B7 (25). However, this interaction does not appear to be essential for the induction of an anti-LCMV cytolytic immune response mediated by CD8 T cells. An explanation could be that CD8 T cells can become fully activated by costimulation through alternative pathways, which are induced by a replicating viral pathogen but not by a syngenic tumor cell. Such secondary signals may be provided by a set of cytokines or adhesion molecules (1, 3, 5, 38).

Immunoglobulin concentrations are reduced in [CD28.sup.-/-] mice. Whereas the amount of IgM is normal, the pattern of IgG subclasses in CD28-deficient mice is altered. The nature of the changes in T cell-dependent IgG subclasses in [CD28.sup.-/-] mice could lie in an altered pattern of cytokines that are involved in Ig isotype regulation (39). Disruption of CD28-B7 interaction with soluble CTLA4Ig suppresses T cell-dependent antibody responses to sheep erythrocytes or keyhole limpet hemocyanin (22). To investigate whether these results are due to impaired T

or B cell function, we infected CD28 mutant mice with VSV. Our data show that B cell responsiveness is unaltered in the absence of CD28, as indicated by the normal titers of neutralizing anti-VSV IgM, which are independent of T cell helper activity (34). However, the class switch to IgG, which is dependent on the function of T helper cells (34), was significantly reduced, suggesting that T help is diminished in the absence of costimulation by CD28.

In conclusion, our data indicate that CD28 costimulation is differentially required for cell-mediated and humoral immune responses in vivo. The CD28-deficient mice will be a valuable tool to further elucidate the role of costimulatory events by CD28-dependent and -independent mechanisms in the generation of immune responses against pathogens and tumors as well as in the course of autoimmune diseases. The study of these animals could help determine where immunosuppression by disruption of CD28-B7 interaction can be effective as a treatment strategy as well as when T cell activation is dependent on other mechanisms of costimulation.

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17 May 1993; accepted 21 June 1993

7/7/14 (Item 1 from file: 351)
DIALOG(R)File 351:Derwent WPI

012520823
WPI Acc No: 1999-326929/199927
Reducing or ameliorating chronic rejection of transplanted tissue in a mammal
Patent Assignee: LI X C (LIXC-I); STROM T (STRO-I); ZAND M (ZAND-I); ZHENG X X (ZHEN-I)
Inventor: LI X C; STROM T; ZAND M; ZHENG X X
Number of Countries: 083 Number of Patents: 006
Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Week	
WO 9922766	A2	19990514	WO 98US23264	A	19981030	199927	B
AU 9912974	A	19990524	AU 9912974	A	19981030	199940	
EP 969867	A2	20000112	EP 98956450	A	19981030	200008	
			WO 98US23264	A	19981030		
MX 9906105	A1	20000101	MX 996105	A	19990629	200115	
AU 744302	B	20020221	AU 9912974	A	19981030	200223	
AU 200242364	A	20020725	AU 9912974	A	19981030	200260	N
			AU 200242364	A	20020517		

Priority Applications (No Type Date): US 9763853 P 19971031; AU 200242364 A 20020517
Patent Details:

Patent No	Kind	Lan	Pg	Main IPC	Filing Notes
WO 9922766	A2	E	34	A61K-039/395	
Designated States (National): AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW					
Designated States (Regional): AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW					
AU 9912974	A			A61K-039/395	Based on patent WO 9922766
EP 969867	A2	E		A61K-039/395	Based on patent WO 9922766
Designated States (Regional): AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					
MX 9906105	A1			A61K-039/395	

AU 744302	B	A61K-039/395	Previous Publ. patent AU 9912974 Based on patent WO 9922766
AU 200242364	A	A61K-039/395	Div ex application AU 9912974 Div ex patent AU 744302

Abstract (Basic): WO 9922766 A2

NOVELTY - A new method for reducing or ameliorating chronic rejection of a transplanted tissue in a mammalian subject comprises administering to the subject an anti-interleukin 12 (anti-IL 12) antibody.

USE - The method is useful reducing chronic rejection of a transplanted tissue in a mammal (claimed).

pp; 34 DwgNo 0/5

Derwent Class: B02; B04; D16

International Patent Class (Main): A61K-039/395

International Patent Class (Additional): A61K-031/70; A61K-031/71; A61K-031-71; A61K-038/13; A61K-038-13; A61K-039-395; A61K-039/395

7/7/15 (Item 1 from file: 357)

DIALOG(R)File 357:Derwent Biotech Res.

0239815 DBR Accession No.: 1999-09916 PATENT

Reducing or ameliorating chronic rejection of transplanted tissue in a mammal - interleukin-12-specific monoclonal antibody, single chain antibody, humanized antibody, useful for preventing chronic rejection of islet cell transplant

AUTHOR: Li X C; Zand M; Zheng X X

CORPORATE SOURCE: Brookline, MA, USA.

PATENT ASSIGNEE: Strom T 1999

PATENT NUMBER: WO 9922766 PATENT DATE: 19990514 WPI ACCESSION NO.: 1999-326929 (1927)

PRIORITY APPLIC. NO.: US 63853 APPLIC. DATE: 19971031

NATIONAL APPLIC. NO.: WO 98US23264 APPLIC. DATE: 19981030

LANGUAGE: English

ABSTRACT: A method for reducing or ameliorating chronic rejection of transplanted tissue in a mammalian subject using an anti-interleukin-12 antibody is new. The method is used for reducing chronic rejection of transplanted tissue in a mammal. The antibody may be a monoclonal antibody, polyclonal antibody, chimeric antibody, single chain antibody, humanized antibody or fragments or a complementarity determining region- grafted antibody, and is administered in combination with another therapeutic agent which reduces a secondary co-stimulatory immune signal such as soluble CTLA4, B7.1-, B7.2- or CD28-specific antibody. Preferably, the subject is human and the tissue has a mismatch of minor alloantigens with the subject. Particularly, the transplanted tissue is pancreatic islet tissue. (34pp)

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5/AB/16 (Item 3 from file: 351)
DIALOG(R)File 351:Derwent WPI

013718703
WPI Acc No: 2001-202933/200120
XRAM Acc No: C01-060328

Novel human sequence antibody that binds to human cytotoxic T lymphocyte associated antigen-4, useful for inducing, augmenting or prolonging immune response to antigen or for suppressing immune response in patient

Patent Assignee: MEDAREX INC (MEDA-N); HALK E L (HALK-I); KORMAN A J (KORM-I); LONBERG N (LONB-I)
Inventor: HALK E L; KELER T P; KORMAN A J; LONBERG N
Number of Countries: 095 Number of Patents: 005
Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Week	
WO 200114424	A2	20010301	WO 2000US23356	A	20000824	200120	B
AU 200070732	A	20010319	AU 200070732	A	20000824	200136	
EP 1212422	A2	20020612	EP 2000959399	A	20000824	200239	
			WO 2000US23356	A	20000824		
US 20020086014	A1	20020704	US 99150452	A	19990824	200247	
			US 2000644668	A	20000824		
			US 2001948939	A	20010907		
KR 2002047132	A	20020621	KR 2002702451	A	20020225	200280	

Priority Applications (No Type Date): US 99150452 P 19990824; US 2000644668 A 20000824; US 2001948939 A 20010907
Patent Details:

Patent No	Kind	Lan	Pg	Main IPC	Filing Notes
WO 200114424	A2	E	127	C07K-016/00	

Designated States (National): AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
Designated States (Regional): AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TZ UG ZW

AU 200070732	A		C07K-016/00	Based on patent WO 200114424
EP 1212422	A2	E	C12N-015/13	Based on patent WO 200114424

Designated States (Regional): AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI

US 20020086014	A1		A61K-039/395	Provisional application US 99150452
				CIP of application US 2000644668
KR 2002047132	A		C07K-016/18	

Abstract (Basic): WO 200114424 A2
Abstract (Basic):

NOVELTY - A human sequence antibody (I) that specifically binds to human cytotoxic T lymphocyte associated antigen-4 (CTLA-4) which is substantially free of non-immunoglobulin associated human proteins, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a composition (II) of polyclonal antibodies comprising a several (I);

(2) a polyvalent complex (III) comprising at least two (I);

(3) a nucleic acid (IV) encoding a heavy chain of human sequence antibody;

(4) a transgenic non-human animal (V) having a genome comprising a human sequence heavy chain transgene and a human sequence light chain transgene, where an animal has been immunized with a human CTLA-4 (or its fragment or analog), and the animal expresses (I);

(5) a cell line (VI) comprising a B cell obtained from (V);

(6) a hybridoma secreting (I) or its binding fragment, in which the antibody is a:

(a) human sequence comprising heavy chain heavy chain CDR1, CDR2, and CDR3 sequences, SYTMH (S1), FISYDGNNKYYADSVKG (S2), and TGWLGPFDY (S3), respectively, and light chain CDR1, CDR2, and CDR3 sequences, RASQSVGSSYLA (S4), GAFSRAT (S5), and QQYGSSPWT (S6), respectively, and heavy chain and light chain variable region amino acid sequences having a fully defined anti-CTLA-4 antibody 10D1 sequence (S7) derived from VH3-30.3 germline sequence, or a fully defined anti- **CTLA4** 10D1 sequence (S8) derived from VkappaA-27 germline sequence, respectively as given in the specification;

(b) a human sequence antibody comprising heavy chain CDR1, CDR2 and CDR3 sequences, SYTMH (S1), FISYDGSNKHYADSVKG (S9) and TGWLGPFDY (S10), respectively, and light chain CDR1, CDR2 and CDR3 sequences, RASQSVSSSFLA (S11), GASSRAT (S12), and QQYGSSPWT (S6), respectively, and heavy chain and light chain variable region amino acid sequences having a fully defined anti-CTLA-4 antibody 4B6 sequence (S13) derived from VH3-30.3 germline sequence, or a fully defined anti- **CTLA4** 4B6 sequence (S14) derived from VkappaA-27 germline sequence, respectively as given in the specification; and

(c) (I) comprising heavy chain CDR1, CDR2 and CDR3 sequences, SYGMH (S15), VIWYDGSNKYYADSVKG (S16) and APNYIGAFDV (S17), respectively, and light chain CDR1, CDR2, and CDR3 sequences, RASQGISSWLA (S18), AASSLQS (S19), and QQYNSYPPT (S20), respectively, and heavy chain and light chain variable region amino acid sequences having a fully defined anti-CTLA-4 antibody 1E2 sequence (S21) derived from VH3-33 germline sequence, or a fully defined anti- **CTLA4** 1E2 sequence (S3) derived from VkappaL-15 germline sequence, respectively as given in the specification;

ACTIVITY - Cytostatic; immunosuppressive; nootropic; neuroprotective; antiviral; antibacterial; antifungal; antiparasitic; antiinflammatory; anti-HIV (Human Immunodeficiency Virus); antirheumatoid; antiarthritic; antidiabetic; dermatologicalMECHANISM OF ACTION - Immunomodulator; human **CTLA4** -human B7 ligand binding blocker.

The biological activity of (I) (a monoclonal antibody 10D1) was tested in vitro. In order to show that 10D1 binding to CTLA-4 blocks the interaction of CTLA-4 with CTLA-4 ligands, B7.1 and B7.2, competition assays were performed by flow cytometry, fluoroscein isothiocyanate (FITC)-labeled human B7.2-Ig fusion protein was incubated with 58alphabetaCTLA4 T-cells and various concentrations of 10D1 MAb. FITC-labeled **CTLA4** -Ig fusion protein was incubated with murine B7.1 transfected cells and various concentrations of 10D1 MAb (monoclonal Antibodies). The competition assays demonstrate the ability of 10D1 to efficiently inhibit **CTLA4** -B7 interactions at low concentrations (1-10 mug/ml). These in vitro studies demonstrate that MAb 10D1 binds human CTLA-4 with high affinity and specificity and that binding of 10D1 abrogates interaction between B7 co-stimulatory molecules and CTLA-4.

USE - (VII) is useful for inducing, augmenting or prolonging an immune response to an antigen (a tumor antigen or a an antigen from a pathogen) in a patient, where the composition blocks binding of human CTLA-4 to human B7 ligands. The patient is treated with a bispecific antibody which comprises an antibody sequence having an affinity for an antigen from a tumor or a pathogen, a virus (HIV), a bacterium, fungus or a parasite.

The method further involves administering the antigen or its fragment or analog to the patient, whereby the antigen in combination with the human sequence antibody induces, augments or prolongs the immune response. The method also involves administering a cytokine to the patient. (VII) is also useful for treating autoimmune disease in a subject caused or exacerbated by increased activity of T cells and for

treating prostate cancer, melanoma or epithelial cancer. The method further involves administering a tumor cell vaccine, a granulocyte macrophage-colony stimulating factor (GM-CSF) modified tumor cell vaccine or an antigen-loaded dendritic cell vaccine. A polyvalent or polyclonal antibody preparation comprising two (I) is useful for suppressing a immune response in a patient (all claimed).

(I) activates immune responses for treating cancer, infectious diseases and promoting beneficial autoimmune reactions for the treatment of diseases with inflammatory or allergic components. The polyvalent or polyclonal preparations of (I) are useful for treating autoimmune diseases such as rheumatoid arthritis, myasthenia gravis and lupus erythematosus, multiple sclerosis, insulin-dependent **diabetes** mellitus, **transplant** rejection, and inflammation, **graft** versus host disease, by inactivating immune responses. (I) is also useful as a diagnostic reagent in vitro for diagnosing the above mentioned conditions.

pp; 127 DwgNo 0/17

5/AB/17 (Item 4 from file: 351)

DIALOG(R)File 351:Derwent WPI

013228060
WPI Acc No: 2000-399934/200034
XRAM Acc No: C00-120763

Preventing or treating transplant **rejection in a recipient comprises administering a monoclonal antibody specific for interleukin-2 receptor beyond the very early phase following** transplantation

Patent Assignee: NOVARTIS AG (NOVS); NOVARTIS-ERFINDUNGEN VERW GES MBH (NOVS); FEUTREN G (FEUT-I); HOWELL R K (HOWE-I); MARBACH P (MARB-I); ROBERTS A (ROBE-I); SCHREIER A B (SCHR-I); SCHREIER D M (SCHR-I); SCHREIER K (SCHR-I); SCHREIER M H (SCHR-I); SCHULZ M (SCHU-I)

Inventor: FEUTREN G; HOWELL R K; MARBACH P; ROBERTS A; SCHREIER M H; SCHULZ M; SCHREIER A B; SCHREIER D M; SCHREIER K

Number of Countries: 090 Number of Patents: 005

Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Week	
WO 200030679	A1	20000602	WO 99EP8988	A	19991122	200034	B
AU 200013846	A	20000613	AU 200013846	A	19991122	200043	
EP 1131098	A1	20010912	EP 99972545	A	19991122	200155	
			WO 99EP8988	A	19991122		
US 20010041179	A1	20011115	WO 99EP8988	A	19991122	200172	
			US 2001862212	A	20010522		
JP 2002530354	W	20020917	WO 99EP8988	A	19991122	200276	
			JP 2000583562	A	19991122		

Priority Applications (No Type Date): GB 9825632 A 19981123

Patent Details:

Patent No	Kind	Lan	Pg	Main IPC	Filing Notes
WO 200030679	A1	E	17	A61K-039/395	

Designated States (National): AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

Designated States (Regional): AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW

AU 200013846	A		A61K-039/395	Based on patent WO 200030679
EP 1131098	A1	E	A61K-039/395	Based on patent WO 200030679

Designated States (Regional): AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI

US 20010041179	A1		A61K-039/395	Cont of application WO 99EP8988

JP 2002530354 W 17 A61K-039/395 Based on patent WO 200030679

Abstract (Basic): WO 200030679 A1
Abstract (Basic):
NOVELTY - Preventing or treating **transplant** rejection in a recipient of organ, tissue or modified or unmodified cell **transplant** comprises long term administration of a monoclonal antibody specific for interleukin-2 receptor (IL-2R) beyond the very early phase following **transplantation** .
DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a monoclonal antibody specific for IL-2R.
ACTIVITY - Immunosuppressant.
No biological data is provided.
MECHANISM OF ACTION - Interleukin-2 receptor antagonist.
No biological data is provided.
USE - Treat or prevent **transplant** rejection.
ADVANTAGE - The use of a monoclonal antibody specific for IL-2R decreases **transplant** rejection in an immunosuppression-intolerant or -non-compliant recipient or xenotransplant rejection compared with available therapies. Also, undesirable side effects associated with current treatments e.g. renal dysfunction, hirsutism, gingival hyperplasia and hypertension are avoided.
pp; 17 DwgNo 0/0

5/AB/18 (Item 5 from file: 351)
DIALOG(R)File 351:Derwent WPI

012520823
WPI Acc No: 1999-326929/199927
XRAM Acc No: C99-096699
Reducing or ameliorating chronic rejection of transplanted **tissue in a mammal**
Patent Assignee: LI X C (LIXC-I); STROM T (STRO-I); ZAND M (ZAND-I); ZHENG X X (ZHEN-I)
Inventor: LI X C; STROM T; ZAND M; ZHENG X X
Number of Countries: 083 Number of Patents: 006
Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Week	
WO 9922766	A2	19990514	WO 98US23264	A	19981030	199927	B
AU 9912974	A	19990524	AU 9912974	A	19981030	199940	
EP 969867	A2	20000112	EP 98956450	A	19981030	200008	
			WO 98US23264	A	19981030		
MX 9906105	A1	20000101	MX 996105	A	19990629	200115	
AU 744302	B	20020221	AU 9912974	A	19981030	200223	
AU 200242364	A	20020725	AU 9912974	A	19981030	200260	N
			AU 200242364	A	20020517		

Priority Applications (No Type Date): US 9763853 P 19971031; AU 200242364 A 20020517
Patent Details:

Patent No	Kind	Lan	Pg	Main IPC	Filing Notes
WO 9922766	A2	E	34	A61K-039/395	

Designated States (National): AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW
Designated States (Regional): AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW

AU 9912974	A	A61K-039/395	Based on patent WO 9922766
EP 969867	A2 E	A61K-039/395	Based on patent WO 9922766

Designated States (Regional): AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE

MX 9906105	A1	A61K-039/395	
AU 744302	B	A61K-039/395	Previous Publ. patent AU 9912974 Based on patent WO 9922766
AU 200242364	A	A61K-039/395	Div ex application AU 9912974 Div ex patent AU 744302

Abstract (Basic): WO 9922766 A2

Abstract (Basic):

NOVELTY - A new method for reducing or ameliorating chronic rejection of a **transplanted** tissue in a mammalian subject comprises administering to the subject an anti-interleukin 12 (anti-IL 12) antibody.

USE - The method is useful reducing chronic rejection of a **transplanted** tissue in a mammal (claimed).

pp; 34 DwgNo 0/5

5/AB/19 (Item 6 from file: 351)

DIALOG(R)File 351:Derwent WPI

012408814

WPI Acc No: 1999-214922/199918

XRAM Acc No: C99-063300

Inhibiting rejection of transplanted **tissues by** grafting **a bone at a vascularization site to induce an immunological tolerance of the tissue**

Patent Assignee: UNIV EMORY (UYEM-N)

Inventor: ALEXANDER D Z; LARSEN C P; PEARSON T C

Number of Countries: 022 Number of Patents: 004

Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Week	
WO 9912584	A1	19990318	WO 98US18866	A	19980910	199918	B
AU 9895677	A	19990329	AU 9895677	A	19980910	199932	
EP 1015043	A1	20000705	EP 98949333	A	19980910	200035	
			WO 98US18866	A	19980910		
AU 739277	B	20011011	AU 9895677	A	19980910	200171	

Priority Applications (No Type Date): US 9758360 P 19970910

Patent Details:

Patent No	Kind	Lan	Pg	Main IPC	Filing Notes
WO 9912584	A1	E	45	A61L-027/00	

Designated States (National): AU CA JP

Designated States (Regional): AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

AU 9895677	A			Based on patent WO 9912584
EP 1015043	A1	E	A61L-027/00	Based on patent WO 9912584

Designated States (Regional): AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE

AU 739277	B		A61L-027/00	Previous Publ. patent AU 9895677 Based on patent WO 9912584

Abstract (Basic): WO 9912584 A1

Abstract (Basic):

NOVELTY - Inhibiting rejection of **transplanted** tissues by **grafting** bone at a vascularization site so as to induce immunological tolerance of the tissue by a subject is new.

DETAILED DESCRIPTION - A novel method for inhibiting rejection of a **transplanted** tissue in a subject comprises **grafting** bone into the subject at a site that vascularizes the bone **graft** , where the bone so **grafted** comprises stromal cells and marrow cells, the bone so

grafted inducing immunological tolerance of the **transplanted** tissue by the subject, thereby inhibiting rejection of the **transplanted** tissue.

An INDEPENDENT CLAIM is also included for a method of producing stable hematopoietic **chimerism** in a subject comprising **grafting** marrow-containing bone and stromal cells into a site in the subject that vascularizes the bone **graft** .

USE - The method can be used for **transplanting** tissue such as heart, liver, kidney, skin, bone marrow, **pancreas** or **pancreatic islets** (claimed). Bone **graft** **transplantation** may also have potential to restore missing cell populations or immunological functions in hereditary immunodeficiency syndromes (e.g. Bruton's agammaglobulinemia) or to treat hematopoietic diseases characterized by failure of the bone marrow microenvironment such as myelofibrosis without the need for myeloreductive conditioning.

ADVANTAGE - The **grafting** of bone induces long-term immune tolerance and promotes long-term survival of tissue **grafts** of the same donor type as the bone **graft** , without requiring irradiation of the host. The promotion of long-term **graft** survival using fragments of whole bone is superior to that obtained using bone marrow.

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Reducing or ameliorating chronic rejection of transplanted **tissue in a mammal - interleukin-12-specific monoclonal antibody, single chain antibody, humanized antibody, useful for preventing chronic rejection of** islet **cell** transplant

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ABSTRACT: A method for reducing or ameliorating chronic rejection of **transplanted** tissue in a mammalian subject using an anti-interleukin-12 antibody is new. The method is used for reducing chronic rejection of **transplanted** tissue in a mammal. The antibody may be a monoclonal antibody, polyclonal antibody, **chimeric** antibody, single chain antibody, humanized antibody or fragments or a complementarity determining region- **grafted** antibody, and is administered in combination with another therapeutic agent which reduces a secondary co-stimulatory immune signal such as soluble **CTLA4** , B7.1-, B7.2- or CD28-specific antibody. Preferably, the subject is human and the tissue has a mismatch of minor alloantigens with the subject. Particularly, the **transplanted** tissue is **pancreatic islet** tissue. (34pp)